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*PROGRESS REPORT - -
1999*

*CENTER FOR MEDICAL, AGRICULTURAL AND
VETERINARY ENTOMOLOGY*

AGRICULTURAL RESEARCH SERVICE

U.S. DEPARTMENT OF AGRICULTURE



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The Center for Medical, Agricultural and Veterinary Entomology (CMAVE) is the largest USDA-ARS research center devoted to entomology. The overall goal of the research program is to develop integrated management technologies and strategies for insects and other arthropods of agricultural, medical and veterinary importance. This report provides abstracts of research in progress and is not intended for citation in any publication. Reprints of published articles may be obtained by contacting the individual authors.

RECOGNITION and HONORS

The CMAVE was selected as one of the 26 leading centers of innovation in the United States, (Esquire Magazine, November, 1999). The list included laboratories at Harvard University, MIT, IBM, Caltech, Johns Hopkins, among others. The magazine stated that the institutions chosen for the list "aren't only about ideas; they're about the reality that applying and spreading innovations is as important as the innovations themselves." The article stated that the CMAVE "will lead the bio-war against bugs and manage the ecological tensions between insect friendlies and insect foes."

Richard Brenner and his research team were selected for the SERDP (Strategic Environmental Research and Development Program) project of the year for their work on precision targeting and pesticide reduction. SERDP is a major interdepartmental program of the Environmental Protection Agency, the Department of Defense, and the USDA.

David F. Williams was named Entomologist of the Year and Jeffrey P. Shapiro received an Achievement Award for Research by the Florida Entomological Society. John Sivinski served as President and Pat Greany served as Vice-President of the Florida Entomological Society during 1999.

Peggy Zelonka was honored with selection as South Atlantic Area Secretary of the Year for 1999.

STAFF AND ORGANIZATION CHANGES

During the past year, the CMAVE participated in the development of the new Center for Biological Control Research and Education at Florida A&M University in Tallahassee, Florida. Stuart Reitz was added to the CMAVE staff and is now conducting research at FAMU as part of our cooperative research program on biological control of crop pests. Also, Robert Heath has transferred from CMAVE to the ARS laboratory in Miami, Florida where he has been named Research Leader, and will continue his research on chemical communication in insects and the development of fruit fly traps. In addition, we note the retirements of Patrick Greany, M. S. Mayer and Jack Seawright. These scientists are noted for their many contributions in biological control, physiology of behavior and mosquito genetics, respectively.

Herbert Oberlander
Center Director

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H. Oberlander, Center Director

MISSION

The Center conducts research on insects of agricultural, medical and veterinary importance with the goal of achieving control of pest species through the development of environmentally acceptable approaches. Emphasis is placed on developing components and systems for integrated pest management, based upon an understanding of the behavior, physiology and ecology of pest species. Sensitive detection devices that employ semio-chemicals and electronic technology will provide the means for early intervention. Investigations will lead to biological control based on parasites, predators and microbes, and thus provide alternative, biorational tools for managing populations of pest species. Special attention is focused on insect pests of field and horticultural crops, stored products and on arthropod pests of medical and veterinary importance. Protection of humans from arthropods of medical importance is a continuing priority. The scope of the Center's research is national and international and impacts agricultural production, postharvest storage and transport of agricultural commodities, and protection from household and disease carrying arthropods. Research is conducted to meet the needs of state and federal regulatory agencies, the Department of Defense, industry, universities, growers, commodity groups and the public at large.

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This Research Unit describes, analyzes and manipulates insect behaviors that are responsible for visual and chemical stimuli that regulate reproduction, feeding, foraging and migration. Principles of behavior are emphasized, especially reproductive behavior of pest and beneficial insects. Results of this research are applied directly to control programs and technology, such as genetic eradication programs against Mediterranean fruit flies in Central America and Caribbean fruit flies in Florida, and integrated pest management of lepidopterous pests of field and vegetable crops. The satellite laboratory at Florida A&M University in Tallahassee conducts research on biological control of insect pests of vegetable crops.

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This Research Unit investigates the chemical, biochemical and physiological factors that regulate insect behavior and the interaction of insects with plants and other organisms in the environment. The research program focuses on the following major areas: identification, synthesis, and behavioral evaluation of pheromones that regulate mating and other behaviors of important insect pests; identification, synthesis and behavioral evaluation of kairomones and other semiochemicals that influence the foraging behavior of beneficial entomophagous insects; identification, synthesis and behavioral evaluation of plant-produced chemicals that influence the behavior of insects; and elucidation of the biochemical mechanisms that regulate insect pheromone production, release and perception.

Imported Fire Ant and Household Insects

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This Research Unit develops reduced-risk integrated management strategies for cockroaches and their attendant allergens, pest ants, fire ants and, through cooperative agreements, termites. Areas of research include insecticide detoxification mechanisms; spatially-based risk assessment and insect behavioral ecology pertaining to the development of baits, repellents, and biological control agents; population dynamics; sociobiology of insects; bioecology and biodiversity; and pheromone chemistry and chemical ecology.

Mosquito and Fly

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Research in this Unit results in new technology that provides the basis for integrated management of mosquitoes and filth flies. Recipients and end-users of Unit research include livestock producers; animal, public, and vector abatement organizations; military personnel; and the public. Specific areas of research include: the biological control of mosquitoes and flies (microbial pathogens, parasites, parasitoids); the regulation of fly populations via manipulation of host attraction, host selection, and blood feeding factors; the development of personal protection technology, including the discovery of new mosquito attractants and repellents; and the discovery and use of genetic, biochemical, and physiological factors as regulating mechanisms for populations of mosquitoes and flies.

Postharvest and Bioregulation

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This Research Unit conducts research on the detection, population estimation and control of stored product insects. New detection tools are developed based on acoustical and electronic methods, as well as chemical ecology. Research approaches to population management include the application of insect behavior, molecular biology, biochemistry and tissue culture to the control of growth and development of these insects.

EXAMPLES OF RECENT RESEARCH

Parasites Smell Success

Parasites and predatory arthropods often prevent plants from being severely damaged by killing herbivores as they feed on the plants. A breakthrough in understanding how these biological control agents locate their insect hosts was achieved with the isolation and identification of a volatile chemical, "volicitin", obtained from the oral secretions of beet armyworm caterpillars. When applied to damaged leaves of corn seedlings, volicitin induced the seedlings to emit volatile compounds that attract parasitic wasps which are natural enemies of the caterpillars.

Fruit Flies Find New Trap Alluring

A trapping system based on a new 3-component lure, has been developed for the Mediterranean fruit fly. It has been tested successfully in seven foreign countries. It was also highly effective during a recent Medfly eradication program in Tampa, Florida. An improved lure and trapping system has also been developed for the Caribbean fruit fly.

Genes on the Move

The ability to insert genes into *Drosophila* suggested opportunities for a new approach to insect control. Progress in this area, however, has been held up by a lack of genetic transposons that would allow scientists to insert genes of choice in other insect species of economic importance. Recent experiments with both fruit flies and moths are showing promise. There is new evidence that a new transposon, *piggybac*, will function in both the Indianmeal Moth and the Mediterranean fruit fly, while another transposable element, *hopper*, was isolated from the Oriental fruit fly.

Taking a Bite out of Fire Ants

When Imported fire ants were introduced into the United States, almost all of their natural enemies remained behind in South America. Efforts to introduce biological control of fire ants have led to the first release in the United States of a South American phorid fly. Fly eggs hatch into larvae in the fire ants, and have the peculiar effect of decapitating the host. Then, the flies complete their development in the severed head capsule until they emerge as adult flies.

Houseflies Succumb to Worms

Adult houseflies that develop from larvae infected with parasitic nematodes lived only half as long as uninfected flies. This nematode species, originally collected from Brazil, has potential as a biological control agent for houseflies because it appears to be host specific and can be raised easily in large quantities. Moreover, because there are few natural enemies that attack flies in the larval stage this nematode may be compatible with other biological control agents.

Eavesdropping on Insects

Highly sensitive methods have been developed for detection of hidden infestations of insects in stored grain based on the sounds that are made as the larvae feed. Field trials indicate practical potential for using a sampling system with sound detectors for quantitative sampling of hidden infestations. In addition, a commercial grain probe trap was modified by incorporating a sensor head with infrared electronics so that insects that enter the trap can be electronically sensed and counted. These detection methods may be applicable to a wide variety of insects.

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**Foreign Agricultural Service (MOU between ARS & FAS)

BEHAVIOR
AND
BIOCONTROL

CRIS - 6615-22000-011-00D--Behavioral Ecology and Management of Crop
Insect Pests with Semiochemicals

CRIS - 6615-22000-013-00D--Insect Biological Control Through
Behavioral and Genetic Manipulation

CRIS - 6615-22000-014-00D--Biological Control Through Artificial
Rearing of Natural Enemies and
Manipulation of Host Plant Resistance

A NOVEL METHOD TO REAR THE DIAMONDBACK MOTH PARASITOID *DIADEGMA INSULARE* IN THE LABORATORY

D. L. Johanowicz¹ and E. R. Mitchell

Objective: One important aspect of an augmentative biological control program is to rear ample numbers of female parasitoids for field releases. The parasitoid *Diadegma insulare* currently is being reared for releases to help manage diamondback moth populations. The current rearing system includes the use of fresh plant material, which can be time consuming and expensive. The objective of the present study is to evaluate a method to rear adequate numbers of *D. insulare* (especially females) without the use of fresh plant material.

Methods: This study consisted of two treatments replicated five times on five different dates. Treatments were plain artificial diet (wheat germ-based artificial diet) versus artificial diet coated with approximately 1 tsp. of cabbage flour immediately prior to the experiments. Artificial diet cakes were infested with diamondback moth eggs approximately 5 days prior to the experiments, maintained at 25° C with constant light. Larvae ranged between late second and early third instar, with 250-350 larvae per diet cake; diet cakes with similar numbers of larvae were used per each paired replicate. Treated and untreated diet cakes were placed on steel mesh platforms in 5 L cylindrical plexiglass containers with an organdy cloth top for ventilation. Cages were placed near a window in the laboratory with an oscillating fan blowing at low speed over the tops of the cages. Four, four-day-old pairs of *D. insulare* were placed in each cage. Wasps were

provided fresh honey and water daily for the duration of the study. After four days, wasps were removed and larvae were allowed to continue feeding on the same diet cake until pupation. The resulting data were compared using the sign test where a positive sign was given to the treatment with the higher value for each given replicate (n=5).

Results: The percent of larvae parasitized on the amended diet cakes ($\bar{x} = 93.4 \% \pm 0.9$) was significantly greater than the percentage on the plain diet ($\bar{x} = 61.8 \% \pm 15.5$) ($p = 0.031$). The percent of daughters produced was not significantly different between the plain diet ($\bar{x} = 49.5 \% \pm 4.2$) and the cabbage flour-amended diet ($\bar{x} = 49.9 \% \pm 1.9$), yet was at an adequate level for our rearing program. The absolute numbers of daughters produced per female was significantly greater in the amended diet treatment ($\bar{x} = 34.7 \pm 3.0$) than in the plain diet treatment ($\bar{x} = 21.6 \pm 6.1$) ($p = 0.031$). In each replicate, *D. insulare* females were observed to land on and parasitize hosts on the amended diet within 45 minutes. No landings were observed on the plain diet; all observed attacks were on larvae crawling on the bottom or sides of the cage.

The results obtained suggest that cabbage flour as an artificial diet amendment may be a method to integrate into a *D. insulare* rearing program. It is relatively easy, inexpensive, and produces adequate numbers of female offspring while minimizing numbers of non-parasitized larvae.

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COMPETITION BETWEEN TWO CABBAGE PEST PARASITOIDS: ARE THEY COMPATIBLE WITHIN A BIOLOGICAL CONTROL PROGRAM?

D. L. Johanowicz¹ and E. R. Mitchell

Objective: The beet armyworm (*Spodoptera exigua*) and the cabbage looper (*Trichoplusia ni*) can be serious pests of cruciferous vegetables, including cabbage, greens, broccoli, and cauliflower. Because of concerns with pesticide safety and control costs, an integrated pest management (IPM) approach, including the use of natural enemies, is under investigation. Natural enemies which may be useful in helping to control these noctuid pests include the parasitoid wasps *Cotesia marginiventris* and *Meteorus autographae*. The objective of this study was to determine whether the two might be compatible if used together in a biological control program by evaluating the level of successful parasitism when these two species are forced to compete for larvae within various size categories.

Methods: Larvae of the beet armyworm were divided into five size categories: size 1 (1-2 mm), size 2 (2-3 mm), size 3 (3-4 mm), size 4 (4-6 mm), and size 5 (6-9 mm). Thirty larvae of each size category (each species is capable of parasitizing at least 30 larvae per day) were presented to one mated *C. marginiventris* female and one mated *M. autographae* female caged together in a modified petri-dish for 24 hours. Larvae were allowed to feed on plant material (collards or pigweed) during the 24 hour period, then after the parasitoids were removed, the larvae were moved to feeding cups where they fed on artificial diet until pupation of the moth or of the parasitoid. Each size category was replicated eight times. The numbers of resulting parasitoids were counted, and the

mean number of each species emerging from each size category was calculated and compared with a t-test.

Results: There were significant differences in the number of each species which emerged from the various size categories (Figure 1). Significantly more *C. marginiventris* emerged from size 1 larvae (16.5 ± 4.0) than *M. autographae* (4.88 ± 4.6) ($p < 0.001$). There was no significant differences in the number of *C. marginiventris* (11.8 ± 5.3) and the number of *M. autographae* (8.0 ± 5.2) which emerged from size 2 larvae. There were significantly more *M. autographae* than *C. marginiventris* which emerged from size 3 larvae (11.1 ± 7.3 vs 3.0 ± 1.9) ($p < 0.01$), from size 4 larvae (18.8 ± 5.9 vs 0.5 ± 0.8) ($p < 0.001$) and from size 5 larvae (0.2 ± 0.4) ($p < 0.001$). These results indicate that there is a niche separation when these two species are in a competitive situation, so that it may be possible for both species to coexist if in the same habitat as long as a variety of larvae sizes are available for oviposition. Studies are planned which test the ability of these two species to coexist over the long term. Results of these studies will help us determine whether releasing both species rather than just one would be appropriate as part of an augmentative biological control program.

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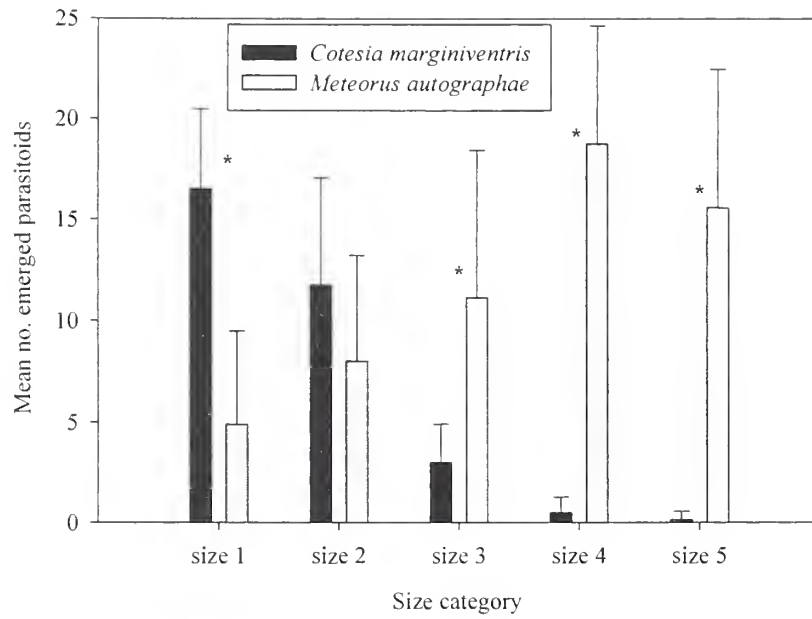


Figure 1. Mean number of parasitoids which emerged from the various size categories of larvae when both species are caged together in a competitive situation. Asterisk indicates significant differences in mean number of larvae.

EFFECTS OF SWEET ALYSSUM FLOWERS ON THE LONGEVITY OF PARASITOIDS THAT ATTACK CABBAGE PESTS

D. L. Johanowicz¹ and E. R. Mitchell

Objective: Conservation of adult parasitoid populations in an agroecosystem so that they may be available at times of increased pest densities is an important consideration in the implementation of biological control programs. Access to nectar may increase parasitoid longevity, and direct provisioning of a carbohydrate source in agroecosystems may help maintain local populations of natural enemies for control of pests. The objective of this study was to assess the effects of sweet alyssum (a hearty winter annual) on longevity of two important parasitoids, *Diadegma insulare* and *Cotesia marginiventris*, in a cabbage ecosystem to determine whether it may be an appropriate flowering plant to include in cabbage fields.

Methods: All studies were conducted in cages located in a glasshouse under ambient light. Ten newly-emerged female parasitoids were housed per cage. The parasitoids were housed with one of the following three treatments: a potted sweet alyssum plant plus a water-soaked cotton ball, honey plus a water-soaked cotton ball, or a water-soaked cotton ball. In the honey treatment, fresh honey was streaked on the cage screen every 2 days. Fresh water was provided to each cage daily. The alyssum was watered daily. Soil-filled pots were added to the cages without sweet alyssum and watered daily to reduce effects of soil or humidity differences. Four replicates of each treatment were conducted for each species.

Cages were monitored daily to record the number of surviving wasps in the cage. The mean number of days until mortality per replicate for each treatment was calculated, an analysis of variance was conducted and the means ($n=4$ per treatment) compared using the Tukey-Kramer HSD means separation test at $\alpha = 0.05$.

Results: The addition of carbohydrate sources had a significant effect on the longevity of *C. marginiventris* and *D. insulare*. The longevity of *C. marginiventris* was significantly different between wasps provided water ($\bar{x} \pm \text{S.E.}$; 4.0 ± 0.2 ; range 2-5 days) and those provided honey (18.6 ± 1.6 ; range 3-33 days) and between wasps provided water and those provided sweet alyssum (19.4 ± 1.1 ; range 4-30 days). There was no significant difference in longevity between wasps provided with honey or sweet alyssum.

C. marginiventris survived on average approximately 4.8 times longer when provisioned with honey or sweet alyssum than with water alone. The longevity of *D. insulare* was significantly different between wasps provided water ($\bar{x} \pm \text{S.E.}$; 2.2 ± 0.3 ; range 1-4 days) and those provided honey (27.2 ± 1.0 ; range 2-51 days) and between wasps provided water and those provided sweet alyssum (27.3 ± 2.7 ; range 1-53 days). There was no significant difference in longevity between wasps provided honey and those provided sweet alyssum. *D. insulare* survived approximately 12.7 times longer when provisioned with honey or sweet alyssum than with water alone.

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COLLECTION OF NOCTUID MOTHS IN SYNTHETIC FLORAL VOLATILE-BAITED TRAPS

R. L. Meagher, Jr.

Objective. Lepidopteran pest populations in agricultural systems are monitored by collecting males in traps baited with synthetically-produced female pheromones. However, chemicals other than sex pheromones have been isolated, identified, and bioassayed as moth attractants. Male and female moth attraction to and feeding from blooming plants has been noted for several pest noctuid species. As early as the late 1920's, the floral compound phenylacetaldehyde was shown to attract various noctuid species in field collections. This chemical and others have been isolated from many flowering plants and shrubs. The objective of these studies was to collect and identify noctuid moths attracted to phenylacetaldehyde and other floral volatiles in field traps.

Methods. Three experiments, conducted in peanut fields, were designed to compare collection of moths in traps baited with different floral volatiles released from different substrates. In the first experiment, standard Unitraps baited with a blend of chemicals (phenylacetaldehyde benzaldehyde, and benzyl acetate) were compared in attraction to traps baited with each component separately. Soybean looper moths, *Pseudoplusia includens* (Walker), were collected and females were dissected to determine mating status.

The second experiment compared collection of soybean looper moths in traps baited with the blend released from glass capillaries or plastic stoppers. The third experiment compared attraction of several noctuid moths to traps baited with phenylacetaldehyde released from plastic stoppers or wax lures.

Results. During the two week period of the first experiment, traps baited with the blend, phenylacetaldehyde, and benzyl acetate collected more soybean looper moths (1462 total, 68% female) than traps baited with benzaldehyde or unbaited traps (Fig. 1). In the second experiment, more soybean looper moths were collected in traps that had the blend released from plastic stoppers rather than glass capillaries (Fig. 2). In the third experiment, males and females of several pest species were collected, including soybean looper, velvetbean caterpillar (*Anticarsia gemmatilis* Hübner), corn earworm, [*Helicoverpa zea* (Boddie)], golden looper [*Argyrogramma verruca* (F.)], and subterranean cutworm [*Agrotis subterranea* (F.)]. Generally more moths were collected in traps baited with phenylacetaldehyde in stoppers, however more *A. gemmatilis* were collected in traps baited with 5% phenylacetaldehyde in wax baskets (Fig. 3). More female soybean looper, golden looper, and subterranean cutworms were collected than males.

Fig. 1

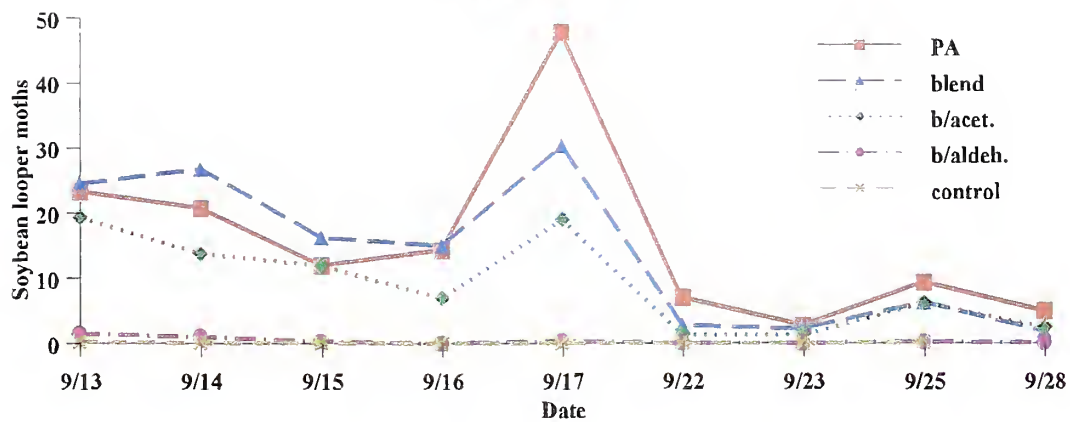


Fig. 2

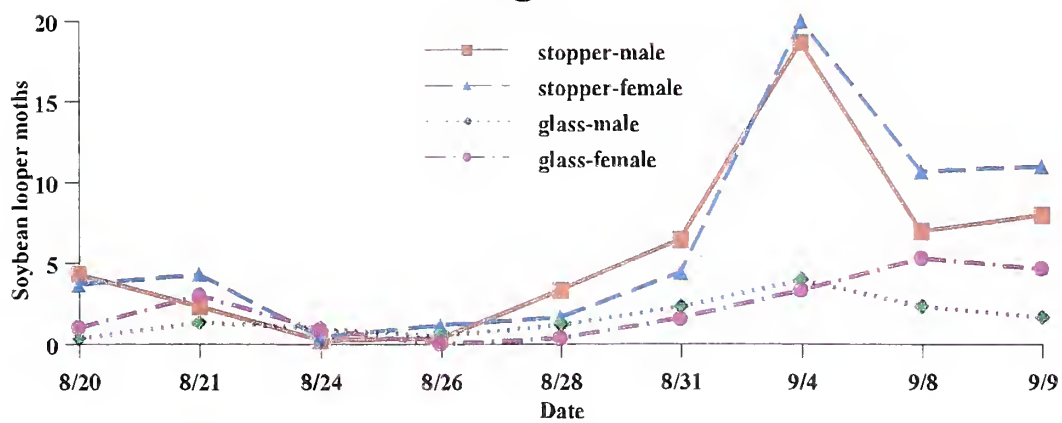
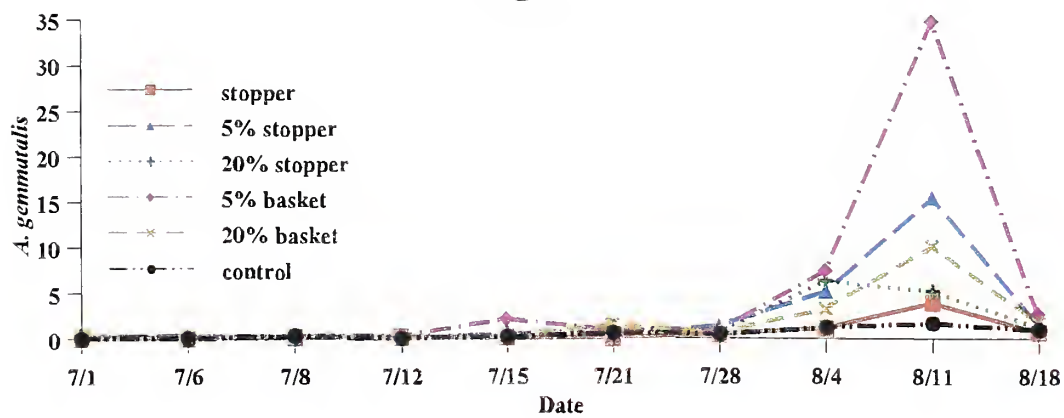


Fig. 3



DEVELOPMENT OF ATTRACT & KILL TECHNOLOGY TO MANAGE BEET ARMYWORM: ATTRACTIVENESS OF LAST CALL FORMULATION

E. R. Mitchell

Objective: To evaluate formulations of Last Call BAW prepared by IPM Technologies, Inc., Portland, OR for attractiveness to wild males when deployed in traps.

Methods: *General Procedure.* All tests comparing lures were conducted using bucket traps with green top, yellow funnel, and white receptacle. A Vaportape® strip was placed in each bucket to kill trapped moths. Treatments were arranged in randomized complete block with 2 or 3 replications/treatment. The traps were mounted on metal poles 1 m above ground and spaced 100' apart within blocks; the distance between blocks was 300'. Traps were inspected at 1-3 d intervals, after which the treatments within blocks were re-randomized. The data were transformed to $\sqrt{X+0.5}$ and subjected to 3-Way ANOVA with days, blocks, and treatments as variables.

Test 1. A 2-component blend of LC BAW without insecticide (LCWO) was evaluated for moth captures at 0.25 and 0.55 g/trap. The LC lures were dispensed from a 3 dram plastic vial stopper (Kimble Glass, Inc.) that had the top insert removed. A Trece rubber septum lure was used as the control. Two replicates(=blocks) of ea treatment were aligned N-S along a field road between 2 corn fields near Palatka, FL, on 01 June 1999.

Test 2. Two- and 3-component blends (both with pyrethroid insecticide) of LC BAW were evaluated for attractiveness to males at Trenton, FL, 09 September-01 October, 1999. Traps were baited with a 0.15 g droplet of LC formulation on a plastic bottle cap or a Trece rubber septum lure. The treatments were deployed E-W in 3 blocks

along a roadway through the middle of a 110 ac, circular cotton field.

Results: *Test 1.* Mean moth captures (\pm s.e) were: 0.25 g LCWO, 122.7 \pm 15.3 a; 0.55 g LCWO, 102.2 \pm 49.5 b; Trece lure, 162.5 \pm 81.9. Means are significantly different, Student-Newman-Keuls method.

Test 2. Mean moth captures (\pm s.e) were: LC 3-component, 9.80 \pm 2.2; LC 2-component, 11.4 \pm 2.8; Trece lure, 6.22 \pm 1.2. There was no statistical significance between mean moth captures for LC or Trece lures.

The results show that LC BAW is attractive to wild males. Although direct comparisons were not made, the results suggest that 1) the dosages of LC BAW used in *Test 1* were too high and 2) the presence of a pyrethroid insecticide in the LC formulations did not affect moth capture. In *Test 2*, both the 2- and 3-component blends (each with pyrethroid insecticide) dispensed at 0.15 g ea captured about the same number of moths as did the Trece lure.

DEVELOPMENT OF ATTRACT & KILL TECHNOLOGY TO MANAGE BEET ARMYWORM: EFFECT OF LAST CALL FORMULATIONS ON SEXUAL COMMUNICATION

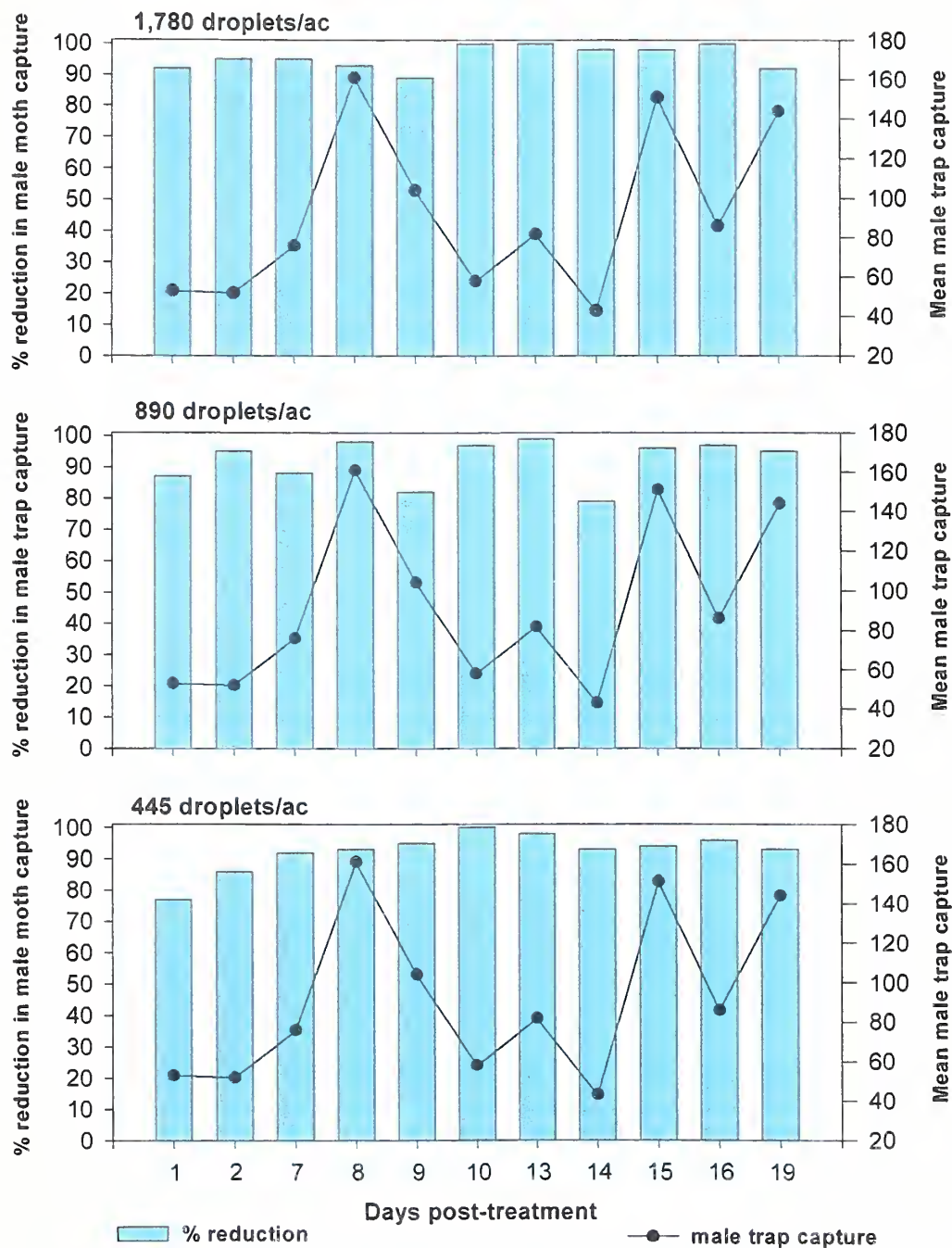
E. R. Mitchell

Objective: To evaluate formulations of Last Call BAW prepared by IPM Technologies, Inc., Portland, OR for capacity to disrupt sexual communication in small field plots.

Methods: Two LC BAW formulations (LCWO and LCWI) were applied by hand to young corn plants on 25 May 1999 at Hastings, FL. at 3 rates: 1,780, 890, and 445 droplets/ac. The dispensers were weighed before and after application to obtain the estimated droplet size (0.55 g ea). Each plot was 35' x 35' (0.028 ac) in size and spaced ca. 200 ft apart in an E-W orientation. Three untreated plots located 300' south of the LC-treated plots served as controls. Treatments were randomly assigned amongst the 6 treatment plots. A bucket trap (green top, yellow funnel, white receptacle) baited with 0.35 g LC formulation in a 3 dram plastic vial stopper (Kimble Glass, Inc.) that had the top insert removed was positioned in the center of each treatment and the 3 control areas. A Vaportape® strip was placed in each bucket to kill trapped moths. Reductions in moth captures compared to the untreated controls served as the measure of treatment efficacy.

Results: Comparison of the numbers of moths captured in traps in plots treated with LCWO and LCWI at the same dosage level showed no significant differences (*t*-test); thus the data for LCWO and LCWI for each treatment level were combined and are presented in Fig. 1. The results indicate that all 3 dosages of LC BAW tested (1,780, 890, and 445 droplets/ac) were highly effective at reducing trap captures of male moths for at least 10 d post-treatment. It is unknown known whether moths were attracted to, contacted the droplet sources, and were killed or incapacitated or they simply were disoriented and thereby unable to find the centrally-located trap in the same numbers as found their way into the control traps. The fact that there was no significant difference in the numbers of moths captured in traps located in plots treated with LCWO and LCWI suggests that disorientation certainly was a factor in reducing moth captures in all plots.

Fig. 1. Percent reduction in capture of male beet armyworm moths in small corn plots treated with Last Call - BAW (0.55 g ea droplet). Hastings, FL. Spring 1999.



DEVELOPMENT OF ATTRACT & KILL TECHNOLOGY TO MANAGE DIAMONDBACK MOTH

E. R. Mitchell

Objective: To evaluate formulations of Last Call DBM prepared by IPM Technologies, Inc., Portland, OR for: 1) attractiveness to wild males when deployed in traps, and 2) capacity to disrupt sexual communication in small field plots.

Methods: *Comparison of LC DBM lures with and without insecticide.* Pherocon 1C sticky traps were baited with LC DBM without insecticide (LCWO)(Lot # CVSI-29A), LC DBM with a pyrethroid insecticide (LCWI)(Lot # CVSI-29B), a Scentry DBM lure on rubber septum, or LC without pheromone or insecticides (control) (Lot # CVSI-30A). The LC products were dispensed (0.5 g ea) into small stainless steel planchets, and placed individually in the center of the trap's sticky bottom. The septum lure also was deposited into a planchet. Treatments were arranged in 3 randomized complete blocks with traps spaced 100 ft apart in an E-W orientation. Blocks were 1,000 ft apart. Traps were set out in heading cabbage at Elkton, FL, on 12 February 1999, and they were inspected every 3-4 days for captured moths. The sticky bottom trap insert was replaced after each inspection. Trap capture data were transformed to $\sqrt{X}+0.5$ and subjected to 2-Way ANOVA. Means were separated using Student-Newman-Kuel's method.

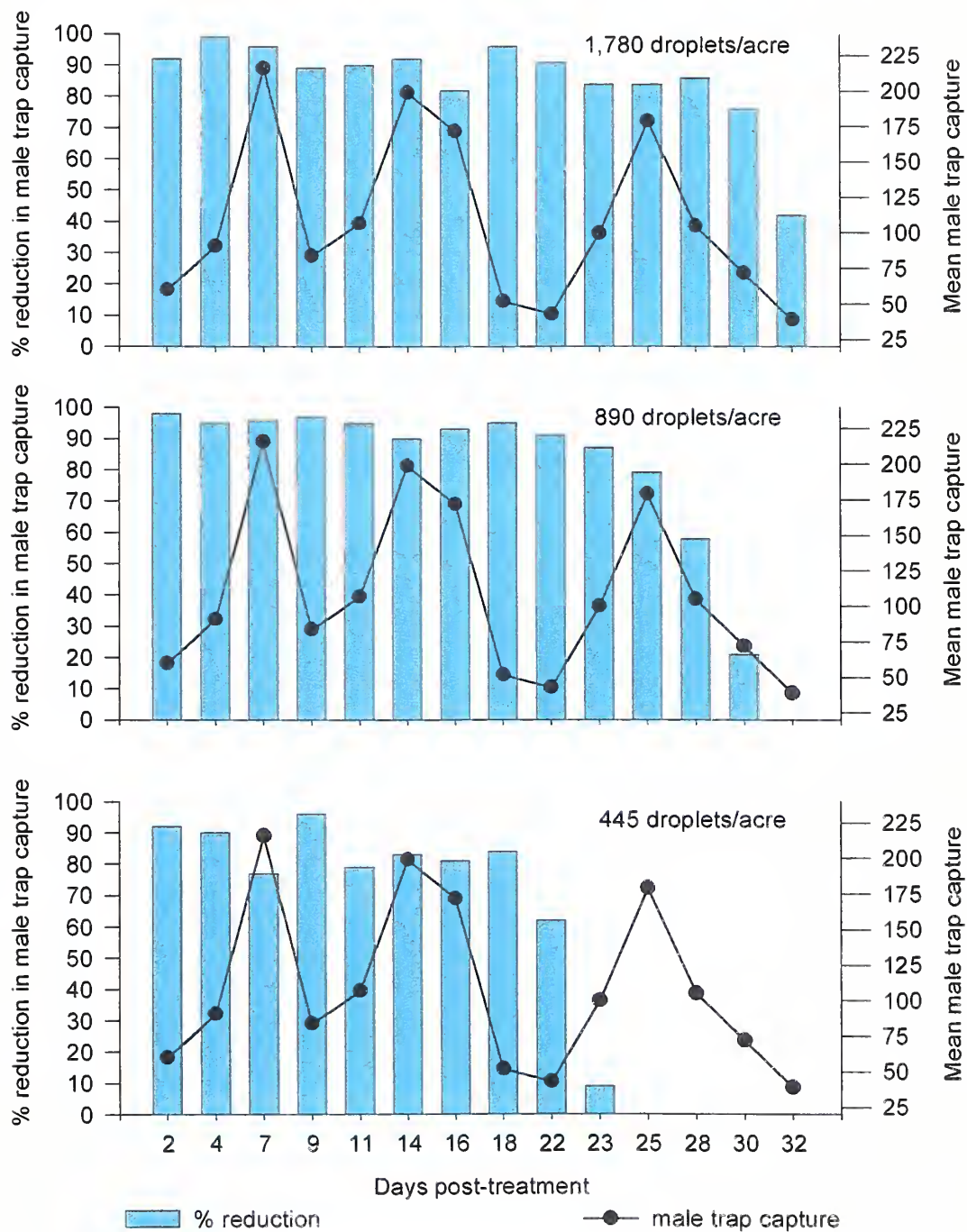
Effect of LC DBM formulations on pheromone communication. Two LC DBM formulations (LCWO and LCWI) were applied by hand to heading cabbage near Bunnell, FL, on 12 April 1999 at 3 rates: 1,780, 890, and 445 droplets/ac. The dispensers were weighed before and after application to obtain the estimated droplet size (0.44 g ea). Each plot was 35' x 35' (0.028 ac) in size and spaced ca. 300 ft apart in an E-W orientation.

Two untreated plots in the line served as controls. Treatments were randomly assigned amongst the 8 plots. A Pherocon 1C sticky trap baited with Scentry DBM lure was positioned in the center of each plot. The numbers of moths captured in traps were recorded every 1-3 days from 04 April-05 May 1999. The sticky inserts were replaced after every inspection. Reductions in moth captures compared to the untreated controls served as the measure of treatment efficacy.

Results: *Lure Comparisons.* Traps baited with LC formulations captured about 40% as many moths as did traps baited with Scentry lures: LCWO, 11.33 ± 2.52 a; LCWI, 10.44 ± 2.38 a; Scentry DBM lure, 26.37 ± 7.44 b; LC control, 0.52 ± 0.44 c. Means followed by different letters were statistically different, $P < 0.001$, SNK method. The LC formulations were attractive to DBM males, and presence of the insecticide did not influence captures.

Effect of LC DBM formulations on pheromone communication. There was no significant difference in numbers of moths captured in LC-treated plots with or without insecticide; hence, the data were combined for each dosage level (Fig. 1). LC DBM reduced trap captures 90% or more for up to 30 d at the highest level tested (1,780 droplets/ac); and the duration of this high level of efficacy was directly related to the number of droplets applied per acre.

**Fig. 1. Percent reduction in capture of male diamondback moths in small cabbage plots treated with Last Call - DBM (0.44g ea droplet).
Bunnell, FL. Spring 1999.**



CORRELATION BETWEEN TEMPERATURE AND LABORATORY BIOLOGY OF *COTESIA MARGINIVENTRIS*

A. Sourakov¹ and E. R. Mitchell

Objective: Our target pest, the cabbage looper (CL), becomes a problem in Florida during the colder months when cruciferous crops such as cabbage and collards are grown. The objective of this study was to test the reproductive performance of the CL parasitoid, *C. marginiventris*, under different temperatures to determine its suitability for augmentative releases.

Methods: Three-four day old beet armyworm (BAW) larvae were offered in clusters of 100 to *C. marginiventris* females at 10, 15, 20, and 25°C. After 24 h, the wasps were removed and the host larvae were reared through on artificial diet in order to determine parasitism. Most host larvae were reared at 25°C, but some were raised at different temperatures to determine rate of development for *C. marginiventris*. BAW larvae were examined daily and the ones with a parasitoid-made exit hole were removed and counted. Similar experiments were conducted using CL as a host.

Results: At 10°C no parasitism occurred; wasps proved to be inactive at this temperature. Parasitism occurred at 15°C and higher. The highest parasitism, was found at 25°C and was equal to 62, yet even at 15°C as many as 43 larvae were parasitized. The wasps were offered the host larvae for only 24 h, so these figures do not illustrate their maximum fecundity (our tests show that females remain fecund for at least three days). Overall, the number of cocoons obtained per female wasp varied greatly and did not correspond to temperatures. In the

BAW experiment, the development time to the cocoon stage at 20°C was 17.6 ± 1.9 days. The time of development was practically the same in younger and in older hosts. In CL the development occurred a little faster than in BAW: 16.1 ± 1.8 days. Cocoon to adult development took 6.6 ± 1.0 days. At 25°C, development occurred much faster: in BAW it took 8.4 ± 0.8 days. Unlike the 20°C set up, at 25°C the parasitoids developed slightly slower inside CL: 9.7 ± 1.0 days. Cocoon to adult stage development takes four days at this temperature. Development to the cocoon stage took 48.6 ± 5.8 days at 15°C. *Cotesia marginiventris* cocoons raised at 15°C were brought into 25°C to eclose. Though usually the process takes four days at this temperature, these cocoons did not eclose for two weeks.

This study implies that *C. marginiventris* is not well adapted to cold temperatures. Therefore, the species might be unsuitable for biological control during colder seasons in Florida, if such control requires regeneration of one-time released wasps. The high fecundity at low temperatures suggests that releases of *C. marginiventris* adults would be an effective short-term (pesticide-like) method to suppress CL populations even during the colder months.

The life stages of *C. marginiventris* are illustrated in Figure 1.

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Life cycle of *Cotesia marginiventris*

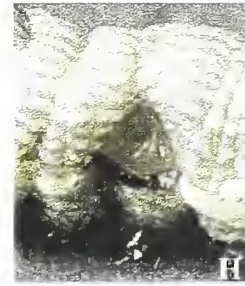
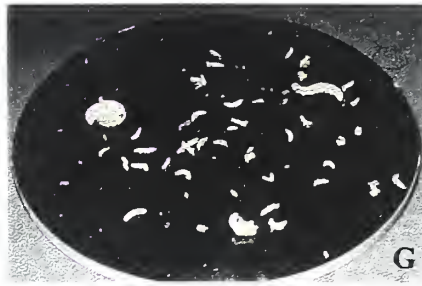
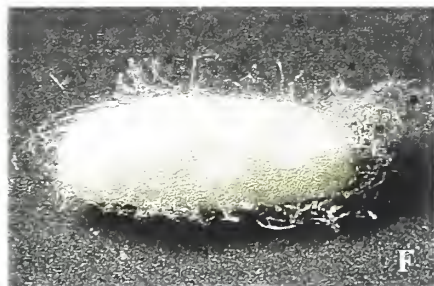


Figure 1. (A) Male of *Cotesia marginiventris*; (B) Mature (4th instar) larva dissected from the host; (C)-(E) Larva emerged from the host and spinning the cocoon; (F) Cocoon; (G) Beet armyworm larvae: large larvae escaped parasitism, while the smaller ones grew much slower and died as a result of parasitism by *C. marginiventris*; (H) An exit hole left in the side of a beet armyworm larva by the emerged *C. marginiventris*.

AN ASSESSMENT OF GREEN FLUORESCENT PROTEIN STABILITY IN TRANSGENIC *ANASTREPHA SUSPENS*A FOR DEVELOPMENT OF GENETICALLY MARKED INSECTS

A. M. Handler and R. A. Harrell II

Objective: To test the stability of green fluorescent protein (GFP) expression from transgenic caribflies killed and maintained in dry trap conditions to assess its ability to function as a genetic marker in released flies.

Methods: The use of GFP marked vectors is important to identifying transgenic fruit flies based upon its fluorescence under ultraviolet light, but also presents the possibility of using GFP as a genetic marker for field detection of released insects. Many of the practical applications for transgenic insects will involve their release, and a common problem for release programs is the necessity to distinguish released from indigenous insects to determine program effectiveness. Trapped insects may not be collected and examined for several weeks, during which time protein degradation and photo bleaching may diminish expression. To test the per durance of GFP expression from transgenic caribflies maintained under dry trap conditions, two-day old adults were killed by decapitation and attached to a plate with double-stick tape in a plastic MacPhail trap. The trap was kept outdoors under partial shade at all times except for daily examination under ultraviolet light. Flies were examined individually under a Leica MZ-12 stereozoom microscope using a mercury lamp and a FITC longpass wavelength filter set. Digital photographic documentation was made with a cooled CCD camera at the same magnification and exposure conditions for all examinations. PCR detection for molecular verification of

transgenic flies used primer pairs based on GFP and *piggyBac* vector sequence. A spectrofluorometric assay for GFP involved homogenization of flies in 10mM Tris pH 7.4, 1mM EDTA 400mM KCl with fluorescence readings taken on the supernatant at 488 nm excitation; 507 nm emission.

Results: Transgenic caribflies from two lines (60 gp1 and 12-4) having four *piggyBac/PUBnlsEGFP* integrations and exhibiting high levels of fluorescence based on spectrofluorometry were tested for visible GFP fluorescence under dry trap conditions. Flies were tested in four groups of five flies each, with two groups of five wild type flies used as controls. The control groups exhibited no detectable fluorescence at any time. Of the transgenic flies, all exhibited fluorescence for at least one week after decapitation, with a significant decrease occurring within three to four days. Ten flies exhibited low, but detectable levels of fluorescence for times ranging from 31 to 68 days after decapitation. These results indicate that enhanced GFP is relatively stable and strains with multiple integrations of the marker can be used as a detection system for released flies using dry traps. Preliminary tests indicate that the gene itself can be detected by PCR in flies dead for extended periods, providing an unambiguous molecular verification of the marked flies. Continued studies will assess GFP per durance under wet trap conditions.

TRANSFORMATION OF *ANASTREPHA SUSPENS*A WITH THE *TRICHOPLUSIA NI* TRANSPOSON VECTOR *PIGGYBAC*, MARKED WITH THE GREEN FLUORESCENT PROTEIN

A. M. Handler and R. A. Harrell II

Objective: To test the ability of the *Trichoplusia ni piggyBac* transposon vector to mediate germline transformation in a tephritid fruit fly, and to test the function of a green fluorescent protein marker (GFP) construct for transgenic selection.

Methods: The *T. ni piggyBac* transposon vector was previously shown to mediate efficient gene transfer in the Mediterranean fruit fly using the medfly *white*⁺ marker and in *Drosophila melanogaster* using a *white* marker and a new green fluorescent protein (GFP) marker. We have now tested the *piggyBac*/PUBnlsEGFP marker in the Caribbean fruit fly, *Anastrepha suspensa*, to determine if 1) *piggyBac* could function similarly in another tephritid fruit fly, and 2) if the GFP marker under *D. melanogaster* polyubiquitin promoter regulation could function in a non-drosophilid species. Embryos from a wild caribfly strain were co-injected with hsp-*piggyBac* transposase helper (phsp-pBac) and a *piggyBac* vector (PUBnlsEGFP) marked with an enhanced GFP gene from pEGFP-1 (Clontech) regulated by the *Drosophila* polyubiquitin promoter that was linked in-frame to the nuclear localizing sequences (NLS) of the SV40 large T-antigen. GFP was observed at all developmental stages under a Leica MZ-12 stereozoom microscope using a mercury lamp and a FITC longpass wavelength filter set (Kramer). Digital photographic documentation used a SPOT-1 cooled CCD camera (Diagnostic Instruments). DNA hybridization was performed by standard methods.

Results: From 1,681 embryos injected 1,089 larvae hatched with 569 surviving to adulthood. The G0 adults were intermated in 60 small mating groups with remaining males group-mated to wild females. Four of the small mating groups yielded a total of 57 G1 fluorescent progeny. Fluorescence was detected in all cells throughout development. Southern DNA hybridization indicated a single integration in two of the G0 lines, with three to four integrations in the other two lines. The *piggyBac* gene transfer vector system has been shown to be effective in another economically important insect, and importantly, the GFP marker system allowed unambiguous selection of transgenic insects. This suggests that this system may be a universal transformation system for economically and medically important insects, and the GFP marker may also be utilized as a genetic marker for released insects.

ECOLOGY AND BEHAVIOR OF TEPHRITID FRUIT FLY PARASITIDS IN MEXICO AND FLORIDA

J. Sivinski and M. Aluja¹

Objective: Biological control sometimes has a major affect on pest fruit fly populations, but in other instances parasitoids either fail to become established or do not flourish and are only rarely recovered. One reason for the failures may be that the wrong parasitoids for the local conditions are being used in control efforts. Parasitoids, including those that attack tephritids, are often specialized. That is, they are active in certain trees, locations, seasons, and times of day. Information on when, where, and how natural enemies hunt for pest fruit flies may help predict which species should be introduced or periodically released in large numbers (augmented) in particular areas.

Methods: In Veracruz State, Mexico the spatial and temporal distributions of braconid, eulophid, eurytomid, eucoilid, and diapriid parasitoids attacking five species of *Anastrepha* fruit flies have been studied for six years. Field samples have been taken to determine altitudinal and regional patterns of abundance. In the laboratory, tests have been used to examine what factors in the environment parasitoids use to locate hosts, and how species with different ovipositor lengths exploit a shared resource. In Florida, the effects on parasitoids of new, supposedly more environmentally safe, insecticides that may be used in fruit fly control have been determined.

Results: Regional sampling has discovered that the abundances and host ranges of various parasitoid species change markedly in different environments. These distributions

are consistent with previous latitudinal and altitudinal transects, and also support findings that host fruit density is an important factor in the survival and increase of certain parasitoids. Since most parasitoids are not as capable of dispersal as their fruit fly hosts, a local variety of trees, fruiting at different times, may provide the best conditions for parasitoids to become established and increase in numbers. A long term experiment where native fruit tree diversity will be re-established near agricultural areas is underway. These trees will harbor nonpest fruit flies that are attacked by the same parasitoids as the pest species. The parasitoids native to southern Mexico have very different ovipositor lengths; presumably this reflects differences in which hosts are attacked and under what circumstances. Comparisons of host ranges, wing structure, and reproductive organs in 5 species of parasitoids have revealed a correlation between ovipositor length and an extremely complex set of morphological and ecological factors. This information will be used to help further predict the "micro habitats" preferred by each species. Using various chemical and visual stimuli, it was determined which fruits are most attractive to a parasitoid commonly used in augmentative releases. The insecticide Spinosad has been suggested as a replacement for Malathion in "bait-sprays" used to suppress and eradicate pest fruit flies. In preliminary field trials, it was found to have little effect on either native parasitoids, a parasitoid introduced into Florida to attack the Caribbean fruit fly, or a family of predaceous flies.

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BIOLOGICAL CONTROL OF MEDFLY IN GUATEMALA

J. Sivinski, T. Holler¹, and F. Jeronimo²

Objectives: The Mediterranean fruit fly is abundant in Central America and threatens to move northward in the Mexico and ultimately the United States. It is prevented from doing so by a sterile fly and pesticide barrier maintained on the Mexican-Guatemalan border by the international organization MOSACMED. However, in recent years this barrier has become increasingly permeable and new techniques are being sought to both improve it and make it more environmentally benign. Biological control has two possible roles in the region: 1) Large numbers of mass-reared parasitoids can be combined with sterile fly releases, and 2) new parasitoids can be introduced that will result in fewer adult flies and so contribute to integrated pest management.

Methods: *Augmented release of parasitoids-* The parasitoid species *Diachasmimorpha tryoni*, *D. kruasii*, and *D. longicaudata* were argumentatively released in a mountainous coffee growing regions of southern Guatemala. Competition among the species will reveal which is the most suitable species for the area. In order to facilitate the aerial release of parasitoids, tests are being made of the effects of chilling (a prerequisite for aerial release) on the fecundity and longevity of the 3 *Diachasmimorpha* species. A pupal parasitoid, *Coptera haywardi*, is being considered for augmentative release. Field cage studies in coffee plantations were carried out to determine its ability to survive and attack medfly hosts under Guatemalan conditions. *Parasitoid introduction-* Explorations for more effective parasitoids for use both in establishment attempts and augmented releases are ongoing in Mexico

and Kenya. The later collections are part of a collaborative effort with the Universities of Hawaii, Florida and Texas A&M, and the International Center for Insect Physiology and Ecology (ICIPE, Nairobi, Kenya). Candidate parasitoids are colonized at USDA-APHIS facilities in Guatemala.

Results: *Augmented releases-* *Diachasmimorpha krausii* appeared to be the best medfly parasitoid of the 3 released species. Chilling had little deleterious effect on the fecundity or longevity of *D. tryoni*. In field cages placed over coffee, *Coptera haywardi* was able to locate and parasitize pupae at depths in the soil of up to 15 mm. It appears to have potential for mass-rearing and augmentative release. *Parasitoid introduction-* Five parasitoids are in colony. These include the first of the new African species, *Psytalia humilis*, and the highly effective egg parasitoid *Fopius arisanus*. In addition, a new pupal parasitoid, an unusual species of Eurytomidae, has been discovered in Mexico where initial colonization is taking place. Plans for a new quarantine facility, the first in Guatemala, have been prepared. In Kenya, rearing experiments with 2 species of *Fopius* collected from medfly have revealed that one, *F. caudatus*, is an egg-pupal parasitoid, and that the other *F. n. sp.* is a parasitoid of first-instar larvae. Parasitoids that attack the very early and vulnerable stages of fruit fly hosts are both rare and potentially very useful in biological control. Funding was obtained to continue the African collections and colonization of these species in Guatemala will be attempted over the following year.

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BIOLOGICALLY BASED PEST MANAGEMENT THROUGH ARTIFICIAL REARING OF NATURAL ENEMIES AND MANIPULATION OF HOST PLANT RESISTANCE

S. M. Ferkovich, J. Shapiro, and J. E. Carpenter

Objective: Effective methods are needed to mass rear high quality beneficial insects that attack pest insects as an alternative to the use of environmentally hazardous pesticides. *Diapetimorpha introita*, a ectoparasitoid that attacks pupae of the army worms, *Spodoptera frugiperda* and *S. exigua*, is a potential candidate for managing these pests, especially in area wide management programs. We are developing an artificial diet for rearing the parasitoid, but improvements still are needed as developmental time, weight of adult wasps and fecundity were inferior compared to wasps reared on natural pupal hosts. Our objective was to examine the potential of supplementing an artificial diet with fats extracted from the host to optimize the diet.

Methods: An artificial diet used to rear *D. introita* was supplemented with lipids extracted from pupae of the host, *Spodoptera frugiperda* (J. E. Smith). Pupae were homogenized and extracted with chloroform:methanol (2:1 v/v) and after drying down the chloroform and methanol phases separately, the residues from each solvent phase were evaluated in the artificial diet. The diet also was sequentially supplemented with four fatty acids (arachidonic, linoleic, γ -linolenic and oleic), flax oil and Lipid Concentrate® which is used in cell culture. After adding the lipids to the diet, it was then encapsulated in paraffin domes and newly hatched parasitoids were each placed on a diet dome and allowed to develop to the adult stage.

Results: Growth-promoting activity was observed in the chloroform phase containing lipids. Diet supplemented with lipid stored at -80°C , and insects reared on diet with fresh 1X and 2X extracts developed significantly faster than those reared on the artificial diet but slower than those reared on host pupae (Table 1). The fresh 1X and the 2X extracts also enhanced the average weight of the males and females, respectively. Storing the lipids at -20°C resulted in a loss of activity. A lipid extract from *Galleria mellonella* pupae increased the average weight of male and females but did not increase their developmental rate. Adult emergence was not improved by any of the dietary additives. None of the commercial lipid treatments significantly reduced developmental time; however, the γ -linolenic acid-supplemented diet significantly increased the average weight of females (Table.2). TLC analyses of the lipid extract from *S. frugiperda* revealed lipids representing four classes of neutral lipids in the extract: triolein, cholesterol, diacylglycerol, and phospholipid. These results suggest that future research should focus on isolating and identifying the active component of the lipid extract in order to find a substitute for the insect lipid(s) that can be added to the artificial diet. Improving the diet will allow production of *D. introita* in numbers that can be used to augment other biorational measures for control of beet and fall armyworm.

Table 1. Effects of supplementing artificial diet of *D. introita* with lipids extracted from host pupae of *S. frugiperda* and pupae of *G. mellonella*.¹

Treatments	Development days \pm SE	Emergence % \pm SE	Male Weight mg \pm SE	Female Wt. mg \pm SE
<i>host pupae</i> (standard)	15.9 \pm 0.09a	73.8 \pm 5.73a	23.2 \pm 0.47a	42.4 \pm 0.60a
<i>artificial diet</i>	18.6 \pm 0.15z	45.0 \pm 5.36z	20.2 \pm 0.44z	35.5 \pm 0.50z
<i>artificial diet with</i>				
<i>host lipid</i> (- 20°C)	18.5 \pm 0.38z	19.4 \pm 6.91az	19.0 \pm 0.77z	34.2 \pm 1.19z
<i>host lipid</i> (- 80°C)	17.1 \pm 0.10az	51.7 \pm 7.98	22.7 \pm 0.54	38.5 \pm 0.59az
<i>host lipid</i> (1X fresh)	16.9 \pm 0.10az	55.7 \pm 4.45	24.4 \pm 0.50a	37.6 \pm 0.39z
<i>host lipid</i> (2X fresh)	17.9 \pm 0.20az	53.5 \pm 2.36	20.6 \pm 0.41	40.4 \pm 1.53a
<i>Galleria lipid</i> (fresh)	18.2 \pm 0.20z	47.3 \pm 6.40	23.7 \pm 0.70a	43.5 \pm 0.61a

¹ Means followed by the letter "a" are significantly different ($P < 0.05$) from the *artificial diet* treatment; means followed by letter "z" are significantly different from the *host pupae* treatment.

Table 2. Effects of supplementing artificial diet of *D. introita* with commercial lipids.¹

Treatments	Development days \pm SE	Emergence % \pm SE	Male Weight mg \pm SE	Female Weight mg \pm SE
<i>host pupae</i> (standard)	15.9 \pm 0.08a	83.4 \pm 3.81a	24.4 \pm 0.99	40.8 \pm 1.23a
<i>artificial diet</i>	18.7 \pm 0.15z	34.4 \pm 8.27z	21.2 \pm 1.75	32.9 \pm 1.56z
<i>artificial diet with</i>				
<i>LipidConc</i> ®	18.3 \pm 0.38z	33.7 \pm 5.11z	16.0 \pm 1.13z	25.7 \pm 1.58az
<i>flax oil</i>	22.6 \pm 0.52a,z	41.5 \pm 1.92z	17.3 \pm 0.88z	25.5 \pm 1.29az
<i>arachidonic</i>	18.2 \pm 0.42z	31.1 \pm 3.75z	23.0 \pm 4.14	36.5 \pm 2.22
<i>linoleic</i>	18.7 \pm 0.19z	48.9 \pm 1.10z	20.5 \pm 1.29	35.8 \pm 3.37
γ - <i>Linolenic</i>	17.7 \pm 0.41z	52.0 \pm 2.10z	21.5 \pm 1.67	42.0 \pm 1.00a
<i>oleic</i>	19.0 \pm 0.52z	53.9 \pm 6.10z	18.0 \pm 1.87z	32.0 \pm 3.18z

¹ Means followed by the letter "a" are significantly different ($P < 0.05$) from the *artificial diet* treatment; means followed by letter "z" are significantly different from the *host pupae* treatment.

DEVELOPMENT OF AN "EARLY PREGNANCY TEST" FOR THE GENERALIST PREDATOR *PODISUS MACULIVENTRIS*, USING ANTI-VITELLOGENIN MONOCLONAL ANTIBODIES IN AN ELISA

J. P. Shapiro

Objective: Egg production (fecundity) in beneficial insects is quantified as oviposition rate or cumulative oviposition. For the predatory stinkbug *Podisus maculiventris*, oviposition may take more than 6 weeks to measure. We are developing a tool to rapidly predict fecundity by quantitatively assessing the reproductive state of females. The test is based on immunoassay (ELISA) of the yolk precursor protein, vitellogenin, in hemolymph. This test may impact the rate of development of artificial diets, provide a tool to monitor product quality in insectaries, and permit rapid assessment of reproductive fitness in augmented field populations.

Methods: Monoclonal antibodies (mAbs) were produced by immunization with an extract of whole eggs from *P. maculiventris*. Two monoclonal hybridomas were selected that reacted strongly against egg extract and female hemolymph, but not against male hemolymph. An antigen ELISA was developed using one of the two mAbs, labeled with biotin to increase sensitivity and detected by Nutravadin conjugated with horseradish peroxidase.

Results: On dot-blots, antibodies from mixed cell cultures reacted strongly against egg extract, less strongly against female hemolymph, and were essentially unreactive against male hemolymph.

When monoclonal cell lines were selected, mAbs showed the same reactivities, and two that reacted the most strongly against female hemolymph were used to develop the ELISA. These mAbs reacted identically on western blots of polyacrylamide gels. Blots of native gels containing female hemolymph proteins showed two bands, one broad and dense and a second one slower moving and narrow. Egg extracts showed one narrow band that moved faster than either hemolymph band. On blots of denaturing (SDS) gels of hemolymph and egg extract, the two mAbs reacted against a single polypeptide band of comparable mobility and a relative molecular weight of 153.

The ELISA developed with these mAbs consistently detected an amount of vitellogenin in blood or vitellin in egg extract equivalent to that in 10-1000 ng of extracted egg protein. Signal was detected in hemolymph diluted to less than 1 ml in 10 ml. This ELISA will be used to describe the dynamics of vitellogenin titers in hemolymph and egg production subsequent to the terminal molt, following ingestion of natural and artificial diets.

EFFECT OF DIET ON REPRODUCTIVE DEVELOPMENT IN *PODISUS MACULIVENTRIS*

H. Dillon-Wasserman¹, P. D. Greany, and J. P. Shapiro

Objective: To expedite diet development by describing reproductive development in adult female *Podisus maculiventris* and developing tests for reproductive response to different diets.

Methods: *P. maculiventris* were fed *G. mellonella* larvae as nymphs, and adults were isolated following the terminal molt. After starving for 5 days, females and males were fed *G. mellonella* larvae or a meat-based artificial diet. Pairs were mated on the eighth day following the molt. Ovarian development was photographically traced, and ovarian follicle lengths were measured. Hemolymph samples were taken at various days preceding and following feeding, and hemolymph samples were applied to electrophoretic gels (SDS-PAGE) to trace the appearance of female-specific polypeptides.

Results: Ovarian development was described morphologically. Following the terminal molt, ovarian maturation score increased from day 2-6 in *G. mellonella*-fed females, while scores of diet-fed females increased only slightly, then fell from day 5-6, and remained low through day 9.

Mean ovariole length increased in *G. mellonella*-fed females from day 7-9, increasing slightly thereafter to ca. 7.7 mm on day 13, while ovariole length in diet-fed females increased at a lower rate, reaching 5 mm at day 13.

Biochemical description using SDS-PAGE showed two female-specific polypeptides in hemolymph, identified as putative vitellogenin subunits. A 153k relative molecular weight (M_r) polypeptide band appeared on day 6 in *G. mellonella*-fed females, and was very dense by day 8. In contrast, a band of the same M_r showed only on day 9 in diet-fed females, and was very dense by day 10. The 153k band was the same M_r as the largest of three polypeptide bands appearing in eggs. A second band in eggs was seen at 130k M_r . It was as dense in eggs as the 153k band, and appeared as a light band in hemolymph of *G. mellonella*-fed females at day 10 and in diet-fed females at day 13.

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POPULATIONS OF FLOWER THRIPS AND THEIR NATURAL ENEMIES IN MIXED PLANTINGS OF TOMATOES AND PEPPERS TREATED WITH DIFFERENT FERTILIZER REGIMES

S. R. Reitz, I. Baez¹, and J. E. Funderburk²

Objective: To determine if increasing nitrogen fertilization results in larger populations of flower thrips in tomatoes and peppers, and if populations of thrips and their natural enemies in tomatoes is affected by the proximity to pepper plants.

Methods: Pepper and tomato plants were transplanted into a field at the University of Florida North Florida Research and Education Center, in Quincy. Each plot consisted of a row of 20 tomato plants, 20 pepper plants, and 20 tomato plants. To analyze the effect of nitrogen fertilization and proximity to the peppers had on populations of thrips and their natural enemies in tomatoes, the experiment was laid out as a split plot design with five replicate blocks. Three fertilizer treatments (sub optimal, optimal, super optimal) were applied to whole plots, and the positions of tomato plants relative to pepper plants were the subplot treatment. On each sample date, five flowers from each location were sampled. Sampling was conducted biweekly from the onset of flowering.

To analyze the effect of nitrogen fertilization on populations of thrips and their natural enemies in peppers, the experiment was analyzed as a randomized complete block design with multiple observations per plot. The 20 pepper plants per plot were divided into five groups of four plants each. On each sample date five flowers were sampled

from each group of pepper plants. Sampling was conducted biweekly from the onset of flowering through the middle of harvest.

The numbers of each species of flower thrips and the predatory bug, *Orius insidiosus*, were counted for each sample. The primary species of pest thrips present in North Florida are *Frankliniella bispinosa*, *F. occidentalis*, *F. tritici*, and *F. fusca*, which was rarely encountered in this study. Because it was not possible to identify thrips larvae to the species level, these were combined into a single group for analysis.

Results: The following results pertain to early season samples prior to the beginning of fruit set. Tomatoes began flowering before peppers. Therefore, availability of pepper flowers may have affected the early season populations. Greater numbers of thrips and *O. insidiosus* were found in pepper flowers than in tomato flowers. Proximity to pepper had no effect on populations in tomatoes. The predominant species of thrips found in both pepper and tomato flowers was *F. tritici* (50% and 54%), with the next most abundant species was *F. bispinosa* (35% and 44%). *Frankliniella occidentalis*, the western flower thrips, made up only 2% of the adult thrips in tomatoes. The nitrogen fertilization level did not affect the number of adult thrips; however greater numbers of immatures were found in optimal and above optimal nitrogen levels.

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² University of Florida

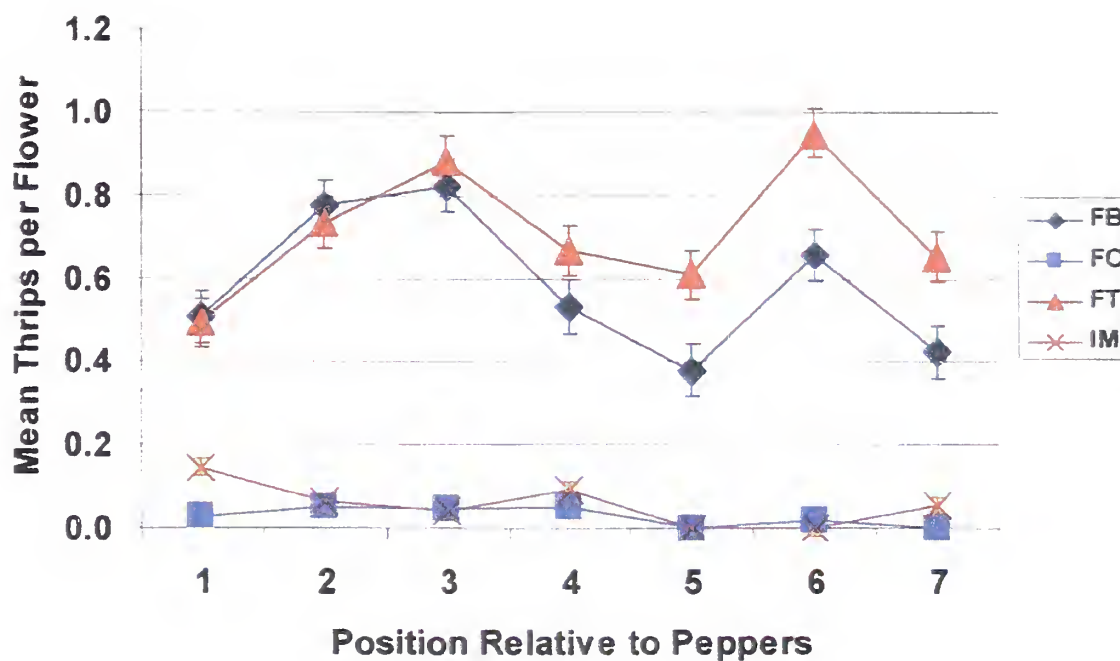


Figure 1. Mean numbers of thrips per tomato flower at each position. Position 1 is closest to the pepper plants; Position 7 is most distant. There were no significant differences among positions for the various thrips. Species are *Frankliniella bispinosa* (FB), *F. occidentalis* (FO), *F. tritici* (FT), immatures of all thrips (IM).

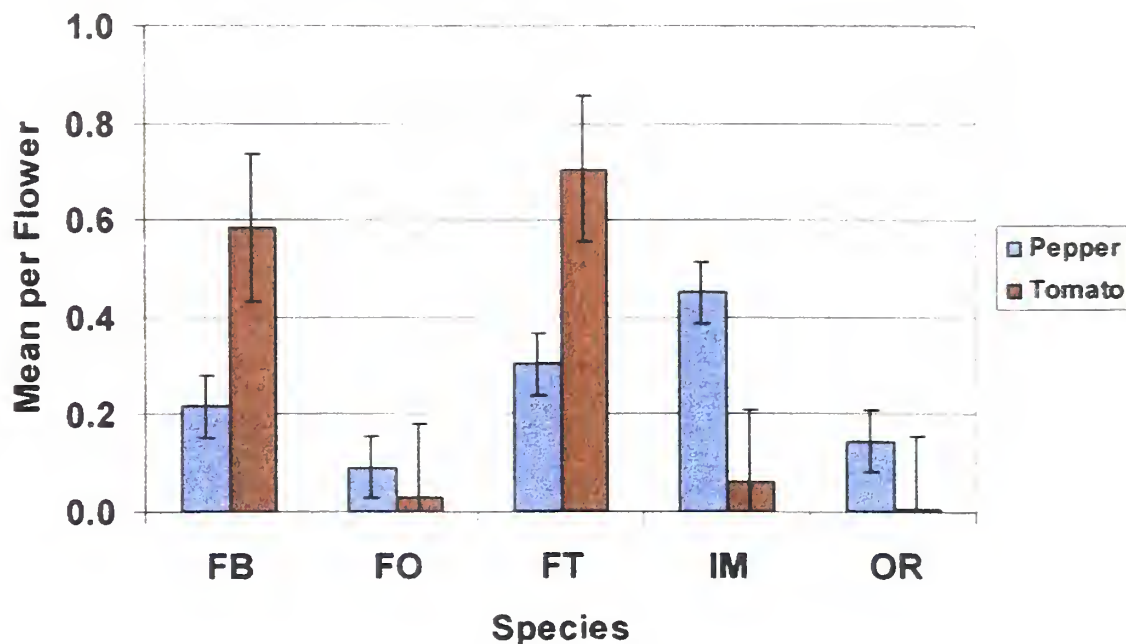


Figure 2. Mean numbers of thrips and *Orius insidiosus* in pepper and tomato flowers. Means and standard errors are presented for all treatments combined. Species designations are the same as in Fig. 1, with the addition of *O. insidiosus* (OR).

CHEMISTRY

CRIS - 6615-22000-012-00D--Chemistry and Biochemistry of Insect
Behavior, Physiology, and Ecology

CRIS - 6615-22000-012-09T-Plant Resistance Induced by Factors in
Insect Herbivore Oral Secretions

CRIS - 6615-22000-012-10R-Mechanism of Detection of Chemical
Signals by Parasitic Wasps

ISOLATION AND IDENTIFICATION OF PLANT VOLATILE ELICITORS FROM *MANDUCA SEXTA* ORAL SECRETIONS

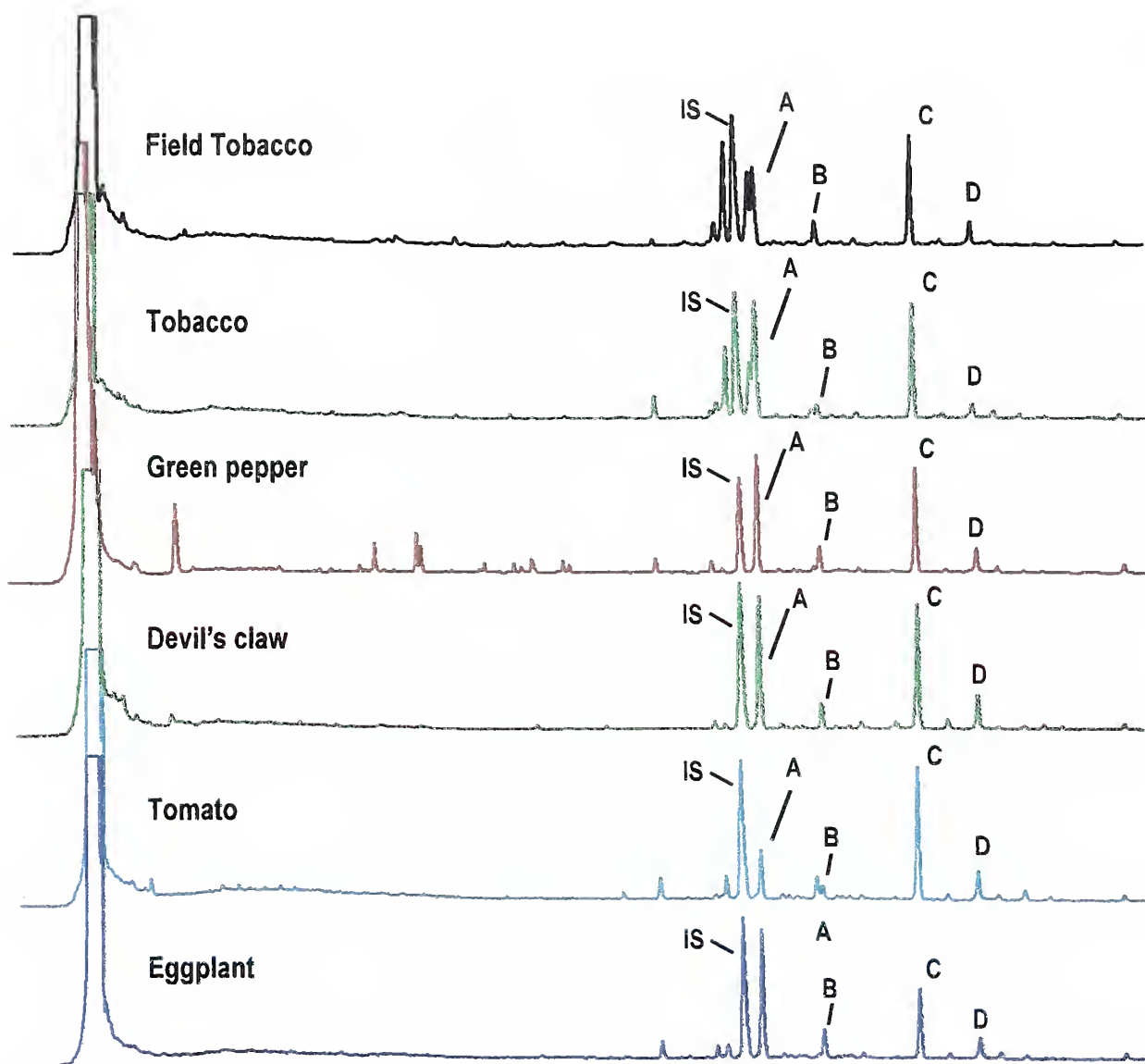
H. T. Alborn, M. M. Brennan and J. H. Tumlinson

Objective: To isolate and identify substances in the oral secretions of *Manduca sexta* caterpillars that induce plants to biosynthesize and release volatile compounds.

Methods: *Manduca sexta* caterpillars were collected from tobacco fields in Alachua County, FL. Laboratory reared larvae were obtained from Raleigh, N.C. and fed on different host plants grown in a greenhouse. Oral secretion was obtained by squeezing the caterpillars and collecting the oral secretion in a vial. Oral secretion is stored at -70 °C. Crude oral secretion is centrifuged at 14,000g for 10 min to remove solids and the supernatant is then filtered through 0.45µm and 0.22 µm sterilizing membranes. Separation and purification of the active compounds is achieved by reverse phase HPLC on a C₁₈ column. Peaks were collected, analyzed for purity, and lyophilized, then methanolysis was performed with subsequent analysis by GC-MS. A bioassay which consists of gas chromatographic analysis of the volatile compounds emitted by corn seedlings is used to monitor fractionation of the oral secretion. An amount of each fraction is added to 500 µl of 50 mM, pH 8, phosphate buffer. A 9 to 11-day-old corn seedling is cut at an angle near the base of the stem with a razor blade and the cut end immersed in the buffer solution in a 1 ml glass vial. The seedling is allowed to draw up the solution over an 17-hr. period. Then one seedling for each treatment is put in a glass volatile collection apparatus (18 cm long, 3.5 cm id) under artificial light. Purified, humidified air

is drawn through the chamber and then through a polymeric adsorbent (Super Q) at 500 ml/min for 2 hr. The adsorbent is extracted with 170 µl of methylene chloride, an internal standard added (400ng/5µl of n-nonyl acetate) and the extract analyzed by capillary GC.

Results: The crude oral secretion of *M. sexta* caterpillars induces corn seedlings to produce and release a similar blend of volatile compounds, but in much smaller quantities than the oral secretion of *Spodoptera exigua* caterpillars, or a solution of its active ingredient, volicitin. Only one active component was found in the *M. sexta* oral secretion. A second, related component was also isolated but showed no biological activity. The active component was identified, as *N*-linolenoyl-glutamic acid, and was synthesized. The elicitor synthesized with L-glutamic acid was as active as the natural product. The active component was not only found in the oral secretions from wild *M. sexta* populations feeding in tobacco fields but also in the oral secretions of laboratory reared *M. sexta* fed on different host plants such as tobacco, green pepper, devil's claw, tomato, and eggplant grown in a greenhouse. Bioassay results of the crude oral secretions from *M. sexta* fed on various plants showed activity similar to that described above (see figure).



A comparison of wild *M. sexta* fed on field tobacco and lab reared *M. sexta* fed on plants grown in a greenhouse. The active component "A" (N-linolenoyl-glutamic acid), the inactive component "B" (N-linoleoyl-glutamic acid), linolenic acid "C," and linoleic acid "D" are found in all oral secretions in approximately the same ratios.

TIMING AND *DE-NOVO* SYNTHESIS OF INDUCED VOLATILE EMISSIONS IN CORN SEEDLINGS

C. M. De Moraes, E. A. Schmelz and J. H. Tumlinson

Objective: The goal of these studies is to understand more clearly how plants, in response to herbivore damage, activate and regulate the synthesis and release of volatile compounds over time. We also intend to establish whether the release of particular volatiles conforms to specific patterns that may be correlated with parasitoid behavior.

Methods: *Plant Growth and Insect Rearing.* Corn plants (*Zea mays* L., var. Delprim), grown from seeds in 16-cm diameter pots using Metromix 300 potting soil, are maintained in an insect-free greenhouse. Eleven-day-old plants are to be used in all studies. Beet armyworms (*Spodoptera exigua* Hübner) are reared on artificial diet by the method of King and Leppla (1984). Third-instar caterpillars are starved for 7 hours before being placed on plants.

Plant Wounding. Two types of experiments will be conducted, live caterpillar damage and artificial damage (with volicitin). For live damage experiments, caterpillars are placed on a plant at the start of the experiment and allowed to feed continuously while the plants are in the volatile collection apparatus (see later section). For artificial damage experiments, mechanically damaged plants are scraped with a razor blade on the underside of the leaf perpendicular to the vein and a volicitin solution or buffer is applied to the leaf at the site of wounding. Total leaf area and insect-damaged portions are measured by scanning a photocopy of the leaves.

Collection and Identification of Volatile Compounds. Collection and identification of

volatile compounds will follow standard procedures developed in our laboratory. Plant volatiles are collected at 1 hour intervals by pulling out half of the air (0.5 l min^{-1}) that has passed over the plant through Super Q adsorbent traps located around the base of the collection chamber; the remainder of the air is vented out the bottom of the system. Temperature and humidity are recorded at 2-minute intervals using a programmable data-logger with built-in sensors. Compounds are eluted from the adsorbent traps with 150 μl of CH_2Cl_2 , internal standards added, and 1 μl aliquots analyzed by capillary GC and GC-MS.

In Vivo Labeling. Intact, growing plants will be labeled with ^{13}C using synthetic premixed air which contains 360 ppm carbon dioxide (^{13}C 99%), 20.7% oxygen and a balance of nitrogen introduced into the volatile collection apparatus by flushing the chamber at 10 l min^{-1} for 2 min and then reducing the flow to 1 l min^{-1} .

Results:

This project is in its initial phase and only preliminary results have been obtained. These appear to indicate that the release of volatiles conforms to a particular pattern although it is too early to draw specific conclusions about the details of the pattern or its implications for parasitoid behavior.

THE BIOLOGY OF CORN SEEDLING ELICITORS IN *SCHISTOCERCA AMERICANA*

M. E. Donohue, P. E. A. Teal, and J. H. Tumlinson

Objective: To identify tissues that contain elicitors of corn seedling volatiles in *Schistocerca americana* and to provide partial identifications of these elicitors.

Methods: Dissection was performed on four groups of grasshoppers, adult males, adult females, immature males and immature females. The foregut, midgut, hindgut, salivary glands, fat bodies, thoracic muscles, and reproductive systems were removed from all groups. Each tissue group was homogenized in Nathanson's modified saline. The homogenate was centrifuged and the supernatant was saved for cut seedling bioassays. Twelve day old corn seedlings (*Zea mays* L. var. LG11) were cut at soil level and incubated in the 500 ml of a treatment overnight. Treatments included a positive control of previously collected oral secretions, a negative control of Nathanson's modified saline, and each of the separate tissue supernatants. After incubation, corn seedlings were placed in collection chambers and volatiles were collected over a 2 hour period. Volatiles were analyzed and quantified by gas chromatography. All data was normalized and compared by one-way ANOVA and Fishers LSD using SAS. Further investigation into which tissues in the female reproductive system contain the elicitor was conducted through dissection. Mature eggs, ovarioles, calyx, spermatheca, the entire gut,

and oral secretions were collected from mature females. Each tissue group was homogenized in 2 ml of Nathanson's modified saline and centrifuged to remove large solids. Cut seedling bioassays and volatile collections were set up as above. Additional mature eggs were collected and extracted with methanol and acetonitrile.

Results: The foregut from all groups of grasshoppers elicited release of volatiles from corn seedlings. In both groups of males the hindgut elicited volatiles. The elicitor was not present in the fat bodies, thoracic muscles, or salivary glands of any of the grasshoppers. None of the reproductive systems contained the elicitor, except for the mature females. Further investigation into the mature female reproductive system showed that only the mature eggs contained the elicitor. Tests on the egg extract are still being conducted to provide partial identification of this elicitor.

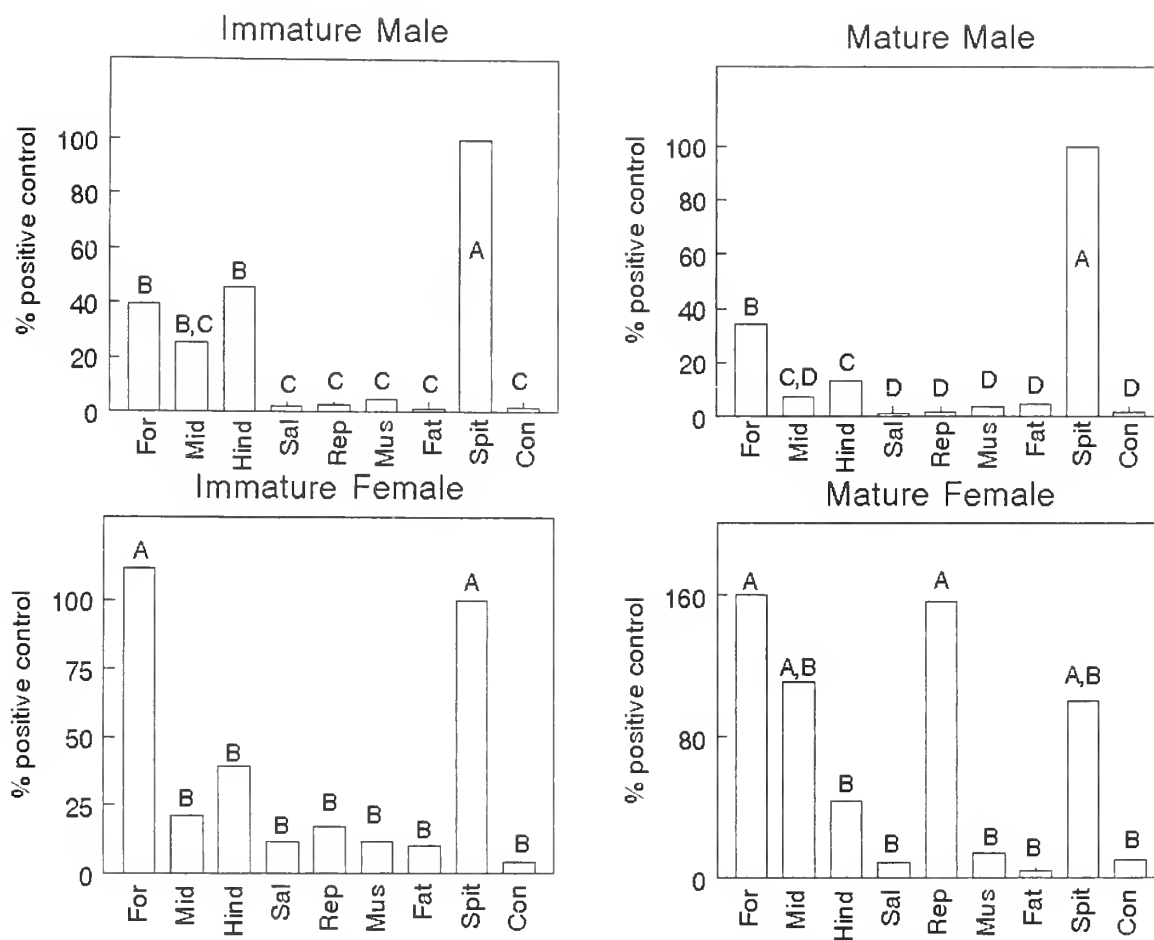


Figure 1. Comparison of individual parts of four groups of grasshoppers. Parts with the same letter are not significantly different ($p < .0239$). For= foregut; Mid= midgut; Hind= hindgut; Sal= salivary glands; Rep= reproductive system; Mus= thoracic muscles; Fat= fat bodies; Spit= oral secretions; Con= control

SMALL HIVE BEETLE (*AETHINA TUMIDA*, COLEOPTERA: NITIDULIDAE) ATTRACTANT

A. Suazo-Calix and J. H. Tumlinson

Objective: The Small Hive Beetle (SHB) was first reported in the United States in July 1998, in Florida, and has since spread to most of the Southeastern states including Georgia, and North and South Carolina. SHB represents a serious threat not only to the beekeeping industry but also to the feral honey bee populations and other social hymenopterans in general (i.e. bumble bees), a major group of insect pollinators. The main objectives of the SHB project are: 1) to determine the mechanisms responsible for the attraction of the beetle to the honey bee colonies, and to apply this information to design traps that could be used to monitor and control beetle populations; 2) to determine the factors that trigger the massive oviposition behavior seen in beetles found in colonies under stress, potentially using this information to control reproduction; and 3) to study basic biological information of the beetle life cycle.

Methods: Honey bee colonies will be established for current and future bioassays and basic behavioral studies. Also, a SHB colony has been established, and beetles are being reared under laboratory conditions as suggested by Lundie. A "dual-purpose" volatile collection system has been developed that can be used to collect volatile compounds from both beetles and honey bees. Also, a four-armed olfactometer has been set up to study beetle behavior.

Results: This project has just begun and no data are available yet.

IDENTIFICATION OF PHEROMONE COMPONENTS IN HEMOLYMPH OF CARIBBEAN FRUIT FLIES

P. E. A. Teal, Y. Gomez-Simuta, and J. A. Meredith

Objectives: To determine the mode of transport of pheromone components in the hemolymph of males of the Caribbean Fruit fly.

Methods: Hemolymph was collected from males and placed in 1ml glass conical vials held in ice. 90 μ l of methanol were added after 10 μ l of hemolymph had been collected and Teflon® lined caps were applied. The samples were vortexed, and 100 μ l of hexane containing 10ng n-tetradecane, as internal standard, were added. Samples were vortexed at 3200 rpm for 2 min. The emulsion was broken by centrifugation at 10,000xg for 5min, and the organic layer was removed with a 250 μ l syringe. The aqueous layer was similarly extracted an additional 2 times with 50 μ l aliquots of hexane. The hexane extract was centrifuged to separate any residual water from the organic extract, and the organic extract was transferred to a clean vial. An additional internal standard, 10ng of (Z)-9-tetradecenoic acid methyl ester, was added prior to concentration to ca. 20 μ l under a gentle stream of N₂, and the sample was analyzed chemically. Initially, we collected hemolymph from sexually mature 12-day-old males during the peak period of sexual activity in the afternoon (2:00-4:30pm). In subsequent experiments we collected hemolymph from 12-day-old males during the early morning period of pheromone production (6:30-7:30am) and during the periods between 8:30-9:30 and 10:30-11:30am when pheromone release is minimal. We also collected samples of hemolymph from males during the peak of afternoon sexual activity on each of the first 8 days after adult eclosion.

Results: Extracts obtained from hemolymph of sexually mature males of the Caribbean fruit fly contained four biologically important terpenoid components of the sex pheromone: farnesene, bisabolene, anastrephin and epianastrepin. The ratio of the components in extracts of hemolymph was the same as the ratio present in the volatile blend of pheromone released by sexually mature males during the reproductive period. Studies conducted to determine the effect of age on amounts of these components in hemolymph indicated that the amounts increased from undetectable amounts on the day of adult emergence to maximum levels on day eight. The increases in amounts of the components present in hemolymph with increasing age were correlated with increases in amounts of volatile pheromone released by males. Time of day studies showed that the amounts of these components in hemolymph followed the daily pattern of release of volatile pheromone components. Other components of the sex pheromone including ocimene, (Z)-3-nonen-1-ol, (Z,Z)-3,6-nonadien-1-ol and suspensolide were not found in extracts of hemolymph.

EFFECT OF JUVENILE HORMONE SUPPLEMENT THERAPY ON MATING AND PHEROMONE PRODUCTION IN CARIBBEAN FRUIT FLIES

P. E. A. Teal, Y. Gomez-Simuta, J. A. Meredith and A. T. Proveaux

Objectives: To determine the effect of juvenile hormone supplement therapy on development and coordination of reproductive competence and sexual signaling in male Caribbean Fruit flies.

Methods: Juvenile hormone (JH) or the JH mimics, methoprene or fenoxycarb were applied at a dose of 5ug to the thorax of males in a 0.5µl drop of acetone on the day of adult eclosion. Controls were treated with just a 0.5µl drop of acetone. Behavioral studies on the interactions between calling behavior and mating were studied in cage bioassays. Treated and control males were caged with females on the day after emergence and observed for calling behavior and mating during the reproductive period of each day until all females had mated. Pheromone production by treated and control males was monitored by collecting volatiles released groups of 5 males on each day after topical application of treatments. The amount of pheromone released was determined by analysis of the volatile collections by capillary GC and GC-mass spectroscopy.

Results: Application of juvenoids to males on the day of eclosion induced precocious reproductive development and development of sexual signaling. The mean age for 100% calling behavior for males treated with JH was 4.7(±0.4) days and those for methoprene and fenoxycarb were 4.0 (±0.0) days whereas the mean age for 100% calling by control males was 7 (±0.2) days. Males treated with methoprene and fenoxycarb released significantly more pheromone per hour on days 3-5 and JH treated males released more pheromone than controls on day 5. While the mean age for 100% mating by control treated males was 6.2 days (±0.6), males treated with JH (4.7 ± 0.5 days), methoprene (4.0 ± 0 days) or fenoxycarb (4.0 ± 0 days) all mated significantly earlier.

IDENTIFICATION OF JUVENILE HORMONES FROM MALES OF THE CARIBBEAN FRUIT FLY

P. E. A. Teal, Y. Gomez-Simuta, J. A. Meredith and A. T. Proveaux

Objectives: To identify homologs of juvenile hormone produced by adults of the Caribbean Fruit fly.

Methods: We collected hemolymph separately from 12-day-old males and mated and virgin 7-day-old males and extracted with hexane containing farnesyl acetate as a quantitative internal standard. The hexane extracts, without further purification, were subjected to GC-chemical ionization (isobutane) mass spectral analysis using a Finnigan-Matt ITS 407 ion trap MS interfaced to a Varian Star 34007 GC. The GC was equipped with a cool-on-column injector. The 30m x 0.25 mm (id) analytical column used in the GC, a DB5-MS7 (J&W), was interfaced to a 10 m x 0.25 mm (id) uncoated, deactivated fused silica retention gap. Conditions of chromatography were: initial injector temperature = 40° for 30 sec; injector temperature increased at 170°/ min to 270°; initial column temperature = 40° for 5 min; column temperature increased at 5°/ min to 210°; He carrier gas linear flow velocity = 24 cm/sec; GC-MS transfer line temperature = 230°. Under these conditions farnesyl acetate eluted at 32.3, JH III at 33.8, and JH IIIB at 34.3 min. Diagnostic ions used for identification and quantification of JH III included m/e = 267 (M+1), 235 (M+1-CH₃OH), 217 (M+1-CH₃OH-HOH), 189 (M+1-CH₃OH-HOH-CO), 147 (M+1-C₂H₄O₂-C₃H₈O) (26). Diagnostic ions used for identification and quantification of JH III bisepoxide (JH IIIB) included m/e = 283 (M+1), 265 (M+1-HOH), 251 (M+1-CH₃OH), 233 (M+1-CH₃OH-HOH), 205 (M+1-CH₃OH-HOH-CO).

Results: Chemical analysis of extracts of hemolymph from 12-day-old virgin males resulted in the identification, for the first time, of JH IIIB as well as its monoepoxide homology (JH III) in a 2.35 ("0.33, n=5): 1 ratio. No other JH homologs were detected. However, we could not detect either JH IIIB or JH III from hemolymph extracts of 1-day-old males. More important, extracts of hemolymph from seven-day-old mated males contained an average of 2.4pg/l ("0.12, n=4) of JH IIIB and JH III. This was significantly more than present in hemolymph from virgins of the same age (0.82 "0.10pg/l, n=4, t=9.331).

DEVELOPMENT OF AMPHIPHYLIC PSEUDOPEPTIDE ANALOGS OF PHEROMONE BIOSYNTHESIS ACTIVATING NEUROPEPTIDE

P. E. A. Teal, R. J. Nachman¹ and J. A. Meredith

Objectives: To design and develop synthetic analogues of insect neuropeptides that penetrate the insect cuticle and maintain bioactivity.

Methods: Pseudopeptide analogs of the C-terminal active core (FSPRLamide) of pheromone biosynthesis activating neuropeptide (PBAN) were synthesized by addition of aliphatic fatty acids to the aminoterminal phenylalanine. Dose response studies of pheromotropotropic activity of analogs were conducted using topical application bioassays in which various concentrations of analogs were applied to the descaled abdomens of females of *Heliothis virescens*. Females were incubated for 1h after injection and then the sex pheromone glands were excised and extracted in hexane containing internal standards. The extracts were then analyzed by capillary gas chromatography to determine the amount of pheromone present. Temporal response studies were conducted by application of 500pmol to the abdomens and incubation for various periods of time prior to excision and extraction of the pheromone glands and chemical analysis of the gland extracts.

Results: The pseudopeptide analogs formed by attachment of acetic, pentanoic, hexanoic, octanoic, decanoic and dodecanoic acids were capable of stimulating pheromone production when applied to females of *H. virescens*. However, an analog formed by attachment of palmitic acid failed to stimulate production of pheromone when applied at doses as high as 2nmol. In dose response studies EC₅₀ values were 10pmol for the acetate analog, 100pmol for analogs formed by attachment of C5, C6, C8 and C10 fatty acids and 500pmol for the C12 analog. Analogs formed by attachment of acetic acid, C5, C6, C8 and C10 fatty acids were equally potent. Temporal activity studies indicated that the acetic acid and C5 fatty acid analogs stimulated pheromone production for as long as 6h. The C6 analog was active for 8h and the C8, C10 and C12 analogs induced production of pheromone for at least 12h. The palmitic acid analog did not induce pheromone production in temporal activity studies.

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ISOLATION AND IDENTIFICATION OF PLANT VOLATILE ELICITORS FROM *SCHISTOCERCA AMERICANA* ORAL SECRETIONS

H. T. Alborn, D. C. Bennett, T. V. Hansen and J. H. Tumlinson

Objective: To isolate and identify the substances in the oral secretions of *Schistocerca americana* that induces plants to biosynthesize and release volatile compounds.

Methods: *Schistocerca americana* adults were obtained from Dr. John Capinera, Dept. of Entomology and Nematology, University of Florida, were maintained under a 14L:10D cycle at 60 % RH and 25 EC, and were fed corn seedlings. Oral secretion was collected from the grasshoppers by gently squeezing them and drawing the oral secretion into a capillary pipette under a slight vacuum. Oral secretion is stored at -70EC. A bioassay that consists of gas chromatographic analysis of the volatile compounds emitted by corn seedlings was used to monitor fractionation of the oral secretion. An amount of each fraction equal to 10 μ l of crude oral secretion was added to 500 μ l of 50 mM phosphate buffer (pH 8). A 9- to 10-day-old corn seedling is cut off above the root with a razor blade and the cut end immersed in the buffer solution in a 1 ml glass vial. The seedling is allowed to draw up the solution over a period of 12 hr in complete darkness. Then the seedling for each treatment is placed in a glass volatile collection apparatus (15 cm long, 3 cm id) under artificial light. Purified, humidified air is drawn through the chamber and then through a polymeric adsorbent (Super Q) at 500 ml/min for 2 hr. The adsorbent is then extracted with 150 μ l of methylene chloride and the extract analyzed by capillary GC. Crude oral secretion is centrifuged at 16,000g for 30 min to remove solids and the supernatant is then filtered through a 0.22 μ m sterilizing membrane. The active compounds are extracted into methylene chloride from a water solution of the oral secretion that has been saturated with a neutral buffer. The methylene chloride extract is evaporated to dryness and re-

dissolved in a neutral buffer. Final purification is achieved by repeated separation on a C18 reverse phase HPLC column using buffered mobile phases at different pH levels.

Results: Compared to the oral secretion of *Spodoptera exigua* caterpillars, the crude oral secretion of *S. americana* induces corn seedlings to produce and release the same volatile compounds in similar proportions, but in approximately 6-fold larger quantities. The active compounds were partially purified by extraction into methylene chloride from a water solution that had been saturated with a neutral buffer. The partially purified components were separated on HPLC using two different water/acetonitrile gradients buffered with ammonium acetate at neutral or acidic pH. The first HPLC separation revealed a group of several similar components, among which only two showed strong activity. Several mg of the active components were collected for identification by repeated injections. These components have been identified using NMR and mass spectrometric techniques.

ENZYMATIC DECOMPOSITION OF INSECT ELICITORS OF PLANT VOLATILES

N. Mori, H. T. Alborn and J. H. Tumlinson

Objective: To establish a method to evaluate tobacco budworm spit enzyme activity for decomposition of volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine] and *N*-(17-hydroxy-linoleoyl)-L-glutamine, which are known elicitors of plant volatiles.

Methods: Corn earworm, *Helicoverpa zea* (CEW), and tobacco budworm, *Heliothis virescens* (TBW), were obtained from Dr. Joe Lewis, USDA/ARS/IBPMRL in Tifton, GA, and were maintained under a 14L:10D cycle at 60 % RH and 25 °C. Early fifth-instar larvae of CEW or TBW, fed on cotton leaves for at least 72 hrs, were dissected in saline under a stereo microscope. The fore-, mid-, and hindgut were then separated and stored at -70 °C. Each gut was homogenized with cold 50 mM phosphate buffer (pH 8), centrifuged at 16,000g for 10 min, and its supernatant stored at -70 °C. The enzyme activity was assayed as follows. The reaction mixture contained 5 µl of either 2.4 mM volicitin (VL) or *N*-linolenoyl-L-glutamine [LN-G(L)] in 50 mM phosphate buffer (pH 8). The mixture was then incubated for 30 min at 24 °C. Each gut homogenate (13-39 µg of protein) was combined with the reaction mixture to yield a total volume of 50 µl assay mixture. The assay mixture was incubated for 60 min at 24 °C and then heated for 30 min at 95 °C to stop the reaction. 5 µl of *N*-palmitoleoyl-L-glutamine solution (1 µg/µl) was added to the assay as an internal standard. The amount of fatty acid moiety released from each substrate was determined by a C₁₈ reverse phase HPLC column with an acetonitrile-water gradient. Protein content was determined according to the BCA protein assay method using bovine serum albumin standards. All assays were carried out in triplicate.

Results: The enzymatic activity that results in the decomposition of volicitin was found to be greatest in the midgut homogenates of both species (Fig. 1). The decreased amount of volicitin, accompanied by increased 17-hydroxylinolenic acid, was due to decomposition by the enzyme from the midgut and contamination of the spit from midgut components. The specific activity in the midgut of TBW appears to be greater than that of CEW. The results showed that proportions of spit components obtained from manually squeezing the caterpillars might not be equivalent to the original proportions to which plants are actually exposed. This might also explain the great variability in quantities of volicitin in beet army worms. It still remains unknown why room temperature decomposition was observed in TBW but not in CEW, despite the fact that the enzyme was contained in the midgut homogenates of both species. With respect to the specificity of the enzyme for VL and LN-G(L), more replications will be necessary to determine significant differences regarding this specificity. In addition, further studies should be done to determine the original proportions of the elicitors without the enzymatic decomposition.

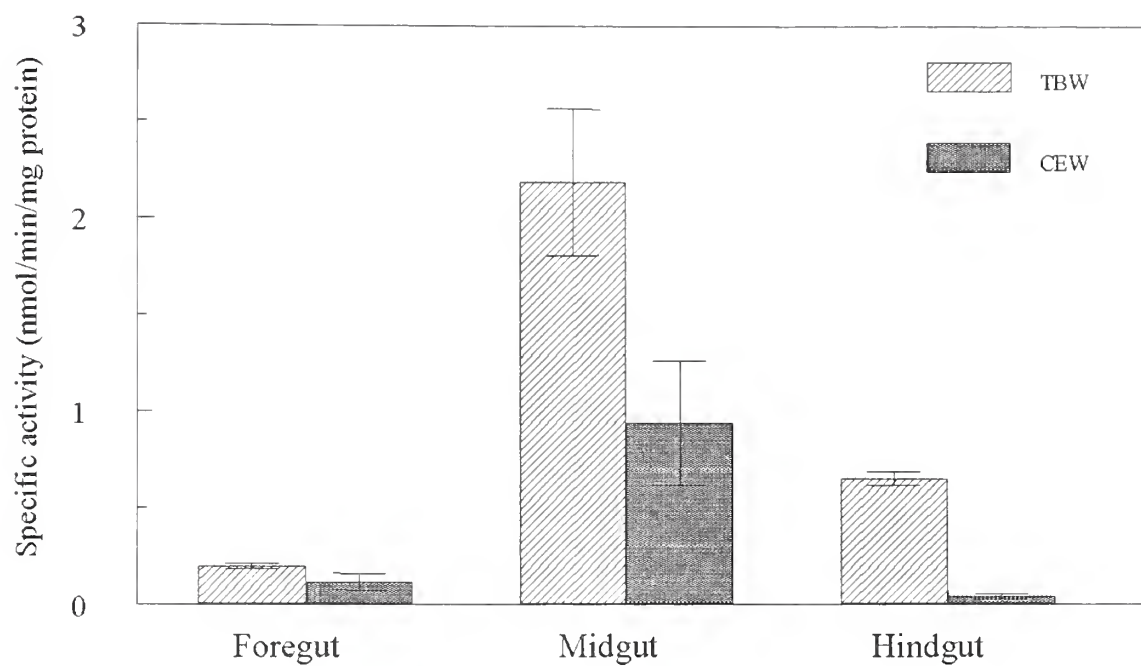


Figure 1. Decomposition of volicitin by the fore-, mid-, and hindgut suspension. All data are shown in mean \pm SEM (N=3).

THE MEDIATION OF PLANT SIGNAL TRANSDUCTION BY INSECT ELICITORS

E. A. Schmelz, C. De Moraes and J. H. Tumlinson

Objective: To determine how the insect elicitor, *N*-(17-hydroxylinolenoyl)-L-glutamine, termed volicitin, interacts with the production of linolenic acid derived wound signals in corn seedlings. Lipid based signaling events that trigger inducible direct defense responses in plants are becoming well established however, the interaction of insect elicitors and endogenous plant signals resulting in elevated volatile biosynthesis (indirect defense responses) is not currently understood.

Methods: Corn seedlings (*Zea mays* L., variety Delprim and LG11) were germinated and grown for 11 days in potting soil. The third leaf, acropetally designated, was excised and used for volatile collection bioassays and fatty acid analysis. Leaf damage treatments employed superficial scraping of the abaxial epidermis with the single pass of a razor blade. One (1mm x 10mm) wound was created on each side of the mid-rib and perpendicular to vasculature. Volicitin and phosphate buffer (50mM, pH 8) treatments were applied directly to the damaged leaf surfaces. Attempts to block the release of free linolenic acid from membrane phospholipids were made utilizing nordihydroguajaretic acid (NDGA), a phospholipase A2 inhibitor. NDGA was dissolved in buffer and applied to the leaves through the base of the cut petiole. Leaves were excised one hour after 'lights off' (12L:12D cycle) and placed into 4 ml vials of buffer. Leaf treatments involving damage and volicitin were performed at least 3 hrs after excision, whereas test compounds in the buffer are added prior to leaf excision (and thus present at time zero). Individual 2 hr volatile collection assays commenced 18 hrs after excision for each

leaf. Identically treated tissues were set up for fatty acid analysis due to the requirement for destructive harvesting. For the isolation of free fatty acids, namely linolenic and linoleic acid, leaves were snap frozen in liquid N₂, lyophilized, extracted in hexane and resolubilized in a minimal volume of acetonitrile. Separation and quantification of the free fatty acids were achieved by HPLC on a reverse phase C18 column, utilizing an acetonitrile/water gradient.

Results: In corn (Delprim) increasing levels of mechanical damage cause proportional increases in the release of volatile sesquiterpenes. At low levels of damage, volicitin greatly enhances the release of this volatile blend. Pre-incubation of leaves with NDGA results in the inhibition of wound-induced volatile production. Likewise, when volicitin and NDGA are applied to the stem, volicitin-induced volatile production is suppressed in a dose dependent manner. At high concentrations, applications of linolenic acid trigger sesquiterpene volatile release similar to nanomole levels of volicitin. Within 14 hrs, volicitin applications result in greater increases in free endogenous linolenic acid and volatile production than mechanical damage alone. This suggests that volicitin changes the amount of free linolenic acid available for the production of wound signals and that this process is a required step of the signaling pathway.

IMPORTED FIRE ANT AND HOUSEHOLD INSECTS

CRIS - 6615-32000-028-00D--Fire Ant Ecology and Management

CRIS - 6615-32000-029-00D--Biological Control of Fire Ants

CRIS - 6615-32000-033-00D--Reduced-Risk Integrated
Management of Medically-
Important Household
Arthropod Pests

FIRE ANT ATTACKS ON RESIDENTS IN HEALTH CARE FACILITIES: A REPORT OF TWO CASES

R. D. deShazo, D. F. Williams, and E. S. Moak

Objectives: Because fire ant densities are increasing, their attacks on animals and humans have also risen. We were concerned about recent fire ant attacks on persons in their own homes, in motels, and in health care facilities. Two attacks on residents in widely separated nursing homes in Mississippi within one year led to this report.

Methods: Clinical records, case series and literature reviews were conducted to obtain the most recent information of indoor fire ant attacks on humans. We reviewed medical records, legal depositions, newspaper reports and other material related to the two fire ant attacks reported here.

Results: A 67-year-old female resident of a skilled nursing home in Brookhaven, Mississippi had dementia, chronic congestive heart failure, chronic obstructive pulmonary disease and osteoporosis. She could not ambulate without assistance and was bed-bound when not in a wheelchair. During a routine bed check at 4:00 am, many fire ants were noted in the bed and all over the patient. More than 500 pustules were present 24 hrs later. Five days later, she died. The other case involved a 60-year-old male resident of a nursing home in Starkville, Mississippi who had developed a right hemiparesis, dysphagia, dysarthria, and incontinence after a cerebrovascular accident 3 years prior to the fire ant attacks. He was attacked in

his bed and had multiple fire ant stings with the characteristic pustules 24 hours later. He was admitted to the hospital the next day. After 21 days of treatment in the hospital for the development of pneumonia and congestive heart failure, he was discharged but never regained the level of function he had before being stung by fire ants. He died 13 months following the attack. With the two incidents reported here, the total number of reported indoor fire ant attacks on humans since 1989 is 10. Six of the persons attacked including the 2 nursing home residents described here (who died after the stings) had preexisting neurologic impairment. Eight of the 10 attacks have been reported in the past 4 years.

CHARACTERIZING THE INTERACTION BETWEEN FIRE ANTS, *SOLENOPSIS INVICTA* (HYMENOPTERA:FORMICIDAE), AND DEVELOPING SOYBEAN PLANTS

R. G. Shatters, Jr. and R. K. Vander Meer

Objective: The fire ant is opportunistic in its resource gathering and takes advantage of a variety of situations where carbohydrates and other food materials are available. This has been documented through above ground fire ant / plant interactions that have been readily observable, and indirectly using radioisotope labeled plants. Here we report the results of experiments designed to study the effect of fire ant association with soybeans during seed germination and plant development.

Methods: Germination studies of soybeans in association with fire ants were performed in large tubs containing soil and fire ant colonies. Four replicates contained fire ant colonies and four replicates were without fire ants. A total of soybean seeds were planted at the end of the tub opposite to the fire ant colony. Seedlings were observed and scored for the number emerged and the amount and type of damage at seven days after sowing. The effect of fire ants on mature plant development was determined as described above, but the experiment was evaluated 90 days after sowing. The number of pods and the fresh weight and dry weight of leaves, stems, pods and roots were determined. The length of the longest root and number of root nodules were also recorded for each plant.

Results: Seedling damage was significantly higher when seedlings germinated in containers containing fire ants. The most notable damage was to the cotyledons. Over 35 % of the ant-associated seedlings had lesions on the cotyledons, whereas, only 2.3% of non-ant associated plants had lesions. The number of delayed emergence

seedlings was significantly higher in fire ant associated plants, a factor used to assess the quality of a given seed lot and to assess seedling vigor. It is apparent that fire ant association during soybean germination reduced the vigor of the developing seedlings.

Fire ant influence on plant development was most notable when plants were grown to the point of seed development in continual contact with the ants. The greatest influence was a 39% and 28% reduction in root fresh and dry weights, respectively. This was associated with a 32% reduction in the length of the root mass in fire ant associated plants as compared to control plants. Nodule formation induced by the nitrogen fixing *Bradyrhizobia bacterium* was reduced by 81% , compared to control plants. The nodules that were visible on the ant-associated plants were shriveled and much smaller than those on control plants, suggesting that they were either non- or poorly functional. Despite the above, biomass of pods produced per plant increased 24 and 43 percent with respect to fresh and dry weights, respectively. The data suggest that some of the biomass produced by the plant was redirected to the developing pods and away from root/nodule development. However, when biomass data from all plant parts are combined, fire ant association still resulted in a 25% loss of total plant fresh weight and an 11% loss of total plant dry weight. The fire ant has an effect on soybean plants throughout their growing cycle.

FIELD HOST RANGE OF THE PARASITIC ANT, *SOLENOPSIS DAGUERREI* IN ARGENTINA WITH REFERENCES TO THE ANT COMMUNITY

L. A. Calcaterra, J. A. Briano and D. F. Williams

Objectives: The workerless, parasitic ant, *Solenopsis daguerrei* has been considered as a potential candidate for the biological control of the imported fire ant, *Solenopsis invicta* in the U.S. The ant occurs only in South America and with the cooperation of ARS's South American Biological Control Laboratory in Buenos Aires, Argentina, our objective is to conduct field research on this insect for it's potential use in the U.S. against *S. invicta*. The specific objective of this project is to determine the field host specificity of *S. daguerrei* and to study some aspects of the ant community.

Methods: The study was conducted in pastures in the area of San Eladio, Buenos Aires Province (60 km west of Buenos Aires) that had the highest abundance of *S. daguerrei* (7 %) in the local fire ant (*S. richteri*) population. Additional surveys were conducted in Las Flores, Buenos Aires Province (190 km southwest of Buenos Aires), and in Colon, Entre Rios Province (300 km north of Buenos Aires). Surveys were conducted from 1996 to 1999. A total of 4,316 ant colonies were visually detected, opened with a shovel and examined for the presence of *S. daguerrei*. Besides visual detection, bait traps were used for additional sampling of the ant populations.

Results: The 4,316 ant nests visually examined plus the bait collections revealed an overall ant's richness of 10 species in 4 subfamilies, however, *S. richteri*, accounted for 96% of the total. *S. daguerrei* was found parasitizing 3.9% (San Eladio), 9.3% (Las Flores) and 12.5% (Colon) of the *S. richteri* colonies at the collecting sites. *S. richteri*, was the only ant species found to be parasitized by *S. daguerrei*. The other species found in numbers were *Pheidole bergi*, *Acromyrmex* spp., *Camponotus punctulatus*, *Neivamyrmex* sp., *Linepithema humile* and *Brachymyrmex* sp. Ants of all species occupied 49% of the bait traps while *S. richteri* accounted for 95% of all ants trapped on baits. These results indicate that *S. daguerrei* is very species specific and although a better understanding of the host and parasite is required, it should be considered as another candidate for use in the biological control of the imported fire ants in the United States.

FIELD RELEASES OF AN ENTOMOPATHOGEN OF IMPORTED FIRE ANTS IN THE UNITED STATES-1999 UPDATE

D. H. Oi and D. F. Williams

Objectives: To infect imported fire ant colonies in diverse geographic locations with the entomopathogen, *Thelohania solenopsae*, and to determine its impact on fire ant populations. This project directly supports the National Fire Ant Strategy, a multi-state effort lead by ARS under the auspices of the Southern Legislative Conference. Goals of this strategy are to develop, test, and implement biologically-based technologies for managing fire ants, and to subsequently develop customized regional integrated management strategies. Originally identified in Brazil in 1973, *T. solenopsae* is the most common pathogen of fire ants in South America. It was discovered in the U.S. in 1996 from fire ant colonies in Florida, Mississippi and Texas.

Methods: Fire ant brood infected with a microsporidian entomopathogen, *T. solenopsae*, was introduced into imported fire ant colonies at paired inoculation and control plots within 10 states (AR, OK, MS, LA, TN, SC, AL, GA, NC, and FL). Baseline data on natural infections of *T. solenopsae* were obtained from worker ant samples. Fire ant populations were assessed by estimating colony sizes using the USDA population index method. The presence of fire ant and non-fire ant species within each plot was determined by counting and identifying foraging ants attracted to a multiple ant species attractant (patent no. 5,939,061) and ants collected in pitfall traps. Post-inoculation evaluations are currently being made at 2 month intervals. Cooperators from state universities, state departments of agriculture, and USDA-APHIS are collecting data for the post-inoculation evaluations. Infection levels are assessed by examining, under phase contrast microscopy (400X), a wet mount

slide prepared from samples of adult worker caste ants from individual colonies, for *T. solenopsae* spores.

Results: Study sites were negative for *T. solenopsae* before inoculations were made. While non-fire ant species were collected from most plots, fire ants were the predominant ant species. *Thelohania* infections have been detected in sites from 7 of the 10 states (AR, AL, FL, GA, LA, MS, NC). Infection rates have been low, and so far, fire ant populations have not yet been significantly impacted.

At another inoculation site in Florida that has been monitored for over 2 years, *T. solenopsae* infections have increased naturally to as high as 89% and fire ant populations have decreased nearly 50% on some sample dates. This is the first site in the U.S. to document some impact associated with *Thelohania*, and more study sites have been established this year.

HOST SPECIFICITY AND RISK ASSESSMENT OF RELEASING THE DECAPITATING FLY, *PSEUDACTEON CURVATUS*, AS A CLASSICAL BIOCONTROL AGENT FOR IMPORTED FIRE ANTS

S. D. Porter

Objective: Host specificity of the decapitating fly *Pseudacteon curvatus* was studied to determine if this species is suitable for release as a classical biocontrol agent of imported fire ants in the United States.

Methods: In order to assess the host specificity of *P. curvatus*, we conducted a series of no-choice tests with variety of native ants and a series of preference tests with native and imported fire ants. We also looked at the attraction of flies to a variety of potential food items.

Results: No-choice tests with 19 species of ants from 12 genera showed that *P. curvatus* will not develop in ants outside the genus *Solenopsis*. *P. curvatus* successfully parasitized the native fire ants *Solenopsis geminata* and *Solenopsis xyloni* in no-choice tests, but rates of parasitism were considerably less than those with the imported fire ant *Solenopsis invicta* (6% and 35% of the rate for *S. invicta*, respectively).

Paired preference tests showed that *P. curvatus* has a 3- to 4-fold preference for *S. invicta* over either of the native fire ants. Furthermore, flies reared from native fire ants still strongly preferred imported fire ants. *P. curvatus* was not attracted to vegetables, fruits, meat, prepared foods, carrion, or dung. This study indicates that release of *P. curvatus* will only pose a small risk to native fire ants. This risk was balanced against potential benefits to numerous other native organisms and a high probability that release of this fly would actually benefit native fire ants because impacts on imported fire ants would almost certainly be much greater than those on native fire ants. Approval to release *P. curvatus* was granted in October 1999.

FIELD RELEASES OF THE DECAPITATING FLY, *PSEUDACTEON TRICUSPIS*

S. D. Porter; L. Alexandre Nogueira de Sá, K. Flanders, L. C. Thompson, C. S. Gorsuch, S. J. Johnson, J. Cook, and J. T. Vogt

Objective: Phorid flies in the genus *Pseudacteon* are a promising group for biological control of imported fire ants. Maggots of these miniature flies develop in the heads of fire ant workers, decapitating their host upon pupation. Fire ant workers are keenly aware of the presence of phorid flies. The presence of a single fly usually stops or greatly inhibits the foraging efforts of hundreds of workers in only a minute or two. The overall impact of these flies on fire ant populations is still unknown; however, it is clearly sufficient to have caused the evolution of a number of phorid-specific defense behaviors. The objective of this study was to determine if the decapitating fly, *Pseudacteon tricuspis*, can survive and proliferate on field populations of the imported fire ant *Solenopsis invicta* in the United States.

Methods: Permits to release *P. tricuspis* were granted to our lab in 1997. Three releases were conducted around Gainesville, FL in the summer and fall of 1997. Flies were released at six additional sites in 1998: Florida (3), Alabama (1), Arkansas (1) and Oklahoma (1). In 1999, we released flies at eight more sites: Florida (2), South Carolina (2), Alabama (1), Louisiana (1), Tennessee (1), and Texas (1). Releases were generally conducted over a 1-3 week period until 1,500-3,000 flies had been released. Each day, we released 30-70 flies over each of 3-6 disturbed mounds. Mounds were disturbed every few minutes for several hours so that flies would have plenty of ants to attack.

Results: Flies have been recovered from one Florida site every month for over 2 years now. Flies have been recovered from three

additional Florida sites almost every month for over one year. Large numbers of flies have been recovered for the last 3-6 months from 4 of the 1999 release sites (Alabama, Florida, and Louisiana, South Carolina). Releases appear to have failed at 6 sites: Florida (2), Arkansas (1), Oklahoma (1), Alabama (1), Tennessee (1). Two releases, one in Texas and one in South Carolina, initially appeared to be successful in the summer of 1999, but no flies have been found at these two sites for the last several months. One release in Florida was completed (Nov. 1999) so it will be several more weeks before we can begin assessing success. So far, our success of establishment appears to be about 50%. Reasons for failures probably include cold winters, polygyne fire ants, summer droughts, and low numbers of flies released at several of the early sites. As of December 1999, fly populations at the 2-year old Florida site have expanded 3-4 miles in all directions. Flies at the three successful 1-year old Florida sites have expanded 1-3 miles in all directions. Currently, decapitating flies in Florida occupy more than 40,000 acres. We will begin assessing the impacts of phorid flies on fire ant populations in the coming year. We will also continue monitoring fly dispersal from the release sites. This information will allow us to determine the value of these flies as fire ant biocontrol agents and how many release sites we will need in each state to achieve maximum benefits in a predetermined amount of time. Decapitating flies will not eradicate imported fire ants in the United States, but they could help reduce their abundance to levels normally found in their South American homelands.

COMPLETION OF 4-YEAR PROJECT UNDER THE STRATEGIC ENVIRONMENTAL RESEARCH AND DEVELOPMENT PROGRAM: PRECISION TARGETING FOR PESTICIDE REDUCTION IN DoD INSTALLATIONS, WAREHOUSING, AND DEPLOYMENTS

R. J. Brenner, D. A. Focks, D. F. Williams, S. M. Valles,
R. T. Arbogast, and P. G. Koehler

Objective: DoD's annual baseline of approximately 1 million lbs of pesticide active ingredients consist principally of insecticides (51%) and herbicides (45%). The project goal is to reduce pesticide use and pollution from pesticides while ensuring control of disease vectors and pests in three major DoD settings: (1) in military deployments and training exercises where risks are from vector-borne diseases; (2) in the DoD supply systems and depots where risks are from damage from stored products pests and other pests; and (3) on military installations where risks of human exposure and collateral environmental damage from pesticides are high.

Methods: USDA/ARS devised a hardware / software "precision targeting" system incorporating ArcView 3.1 GIS. The software allows the collection of spatially-based field data and simple mapping / interpolation functions to visualize extent of infestations and areas that need mitigation. Customized programming allows hand-writing recognition and real-time integration of military or commercial GPS receivers to facilitate data collection, entry, and real-time simplified spatial analysis. The software constructs contour maps that spatially define comparative risks and risk reductions through targeted use of least-toxic pesticides applied only when intervention is necessary.

Results: Field studies were conducted to demonstrate proof of concept on Pharaoh's ants, cockroaches, and stored product pests. In this final year of this 4-yr project, we have focused on providing this system to DoD beta testers, and to

begin transitioning the technology to the Army and Navy entomologists so that they may determine usefulness of the concept in Military Services' pest management programs. Private sector cooperators also have initiated their own field projects. Feedback from these beta testers has resulted in salient changes in the programming to simplify the procedures most useful to pest management practitioners. Programming linkages also have been added allowing access to required DoD pesticide reporting software (IPMIS), and an extensive on-line help / tutorial document has been created to assist users through sequential processes of a typical project. Army entomologists have begun conducting training to extend the use of the software beyond their beta tests. This technology also has received attention from sectors outside DoD; the Pan American Health Organization, Brazil, and Singapore have requested the software for disease vector control. The software will be available after December 31, 1999 through a USDA / ARS website accessible at <http://cmave.usda.ufl.edu/~ifahi>.

VALIDATION OF DENGUE RISK ASSESSMENT ALGORITHMS AND SURVEY METHODS USED IN THE STRATEGIC ENVIRONMENTAL RESEARCH AND DEVELOPMENT PROGRAM (SERDP) SOFTWARE FOR DoD

D. A. Focks, R. J. Brenner, A. Morrison, T. W. Scott, and D. Watts

Objective: Dengue viruses, transmitted between humans by the bite of urban-dwelling *Aedes aegypti* mosquitoes, are endemic throughout most of the tropical and subtropical world. Because dengue vaccines have yet to be developed, prophylactic measures suitable for long-term use by deployed US Armed Forces in endemic locations are limited to suppression of the vector to levels below the transmission threshold. Earlier work related to creation of the SERDP software module for dengue included the development of a rapid risk assessment method, the pupal/demographic survey, and mathematical development of transmission thresholds as a function of *Ae. aegypti* pupae per person, ambient temperature and seroprevalence of dengue antibody. This year, under funding from SERDP and NIH, work was begun in Iquitos, Peru to evaluate and validate the SERDP software risk assessment methods and algorithms for dengue.

Methods: The study, entitled "*Entomological Assumptions of Dengue Control*" is a 5-yr NIH-funded study involving entomologists, virologists, and physicians from the US Navy, UC-Davis, and CMAVE; it is a longitudinal cohort study of dengue in Iquitos to monitor dengue incidence measured at 6 month intervals and entomological risk simultaneously. The primary purpose of the work is to evaluate spatially and temporally the relationships between transmission risk assessment methods and dengue incidence with the goal of developing non-insecticidal control measures based on source reduction- the strategy employed in the SERDP-dengue module. Each year, about 25,000 houses

are exhaustively surveyed allowing the calculation of the various traditional *Stegomyia* and the pupae-per-person indices. Approximately 3,000 spatially-stratified children from these homes are bled and evaluated for dengue seroconversion each year using an initial IgG-ELISA screen followed with dengue serotype-specific neutralization assay. The temporal dynamics and transmission thresholds of dengue are investigated using the dengue transmission models developed by CMAVE; these models will be supplied with the SERDP software. The spatial relationships are evaluated using the SERDP-dengue extension for ArcView 3.1. This initial report presents the observed relationship between the various entomological risk assessment indices and dengue seroprevalence in Iquitos, Peru for the past year.

Results: To date, the entire city of Iquitos (pop. 305,000) has been mapped into an ArcView GIS system. The city has been divided into 8 zones, each subdivided into sub-zones, that vary significantly in entomological indices and seroprevalence of dengue antibody. The baseline serosurvey of 2,488 individuals has been completed with prevalence rising as expected with age. At the sub-zone level, the traditional *Stegomyia* indices, the House, Container, and Breteau, were not correlated with seroprevalence, whereas the pupal indices developed for SERDP were at the 5% level (Pearson's correlation). Both levels of pupae per person and transmission threshold estimates were consistent with theoretical estimates developed and presented last year in this document on transmission thresholds.

DEVELOPMENT OF STANDARDIZED MONITORING TECHNIQUES AND TARGETED BAIT APPLICATIONS FOR FIRE ANTS

D. H. Oi, R. J. Brenner and D. F. Williams

Objective: To develop standardized monitoring and treatment methods to reduce pesticide applications for imported fire ant (IFA) control. Studies were conducted to assess changes in IFA populations after using targeted fire ant bait applications based on the following monitoring techniques: a) fire ant mound locations; and b) the locations of baited ant monitoring stations (MABs) that contained fire ants. Labor (person-hours) was compared between these two methods of monitoring and treating IFA populations.

Methods: For applications based on mound locations, active fire ant mounds were flagged and geo-referenced, or mapped, using a GPS unit. Areas encompassing groups of active fire ant mounds plus about a 25 foot area beyond the outermost fire ant mounds were marked to target fire ant bait applications. Similarly, areas encompassing MABs with fire ants were also mapped and designated for treatment. (MABs were set 50 ft. apart in a grid pattern.) Broadcast bait applications of either Amdro or Award (Logic), using a manual chest seeder calibrated at 1 lb per acre, were made within these areas. Person-hours were calculated per area surveyed and treated to compare labor resources expended. Using spatial surface generators available on Arc-View GIS software, maps were generated to determine how many ants in the MABs would best represent fire ant mound locations.

Results: Fire ant bait applications that were based on mound and MAB locations with fire ants were reduced by 70 and 63%, respectively, when compared to a standard broadcast bait application over an entire area. There was a 55% decrease in person-hours per area used in the targeted treatment method, based on both mound and MAB monitoring, when compared to a standard treatment method of locating and treating individual fire ant mounds. While targeted bait applications may result in a reduction in pesticide use and labor time, fire ant population reductions were at most 52%, and thus not acceptable. Because these locations were heavily infested with fire ants, a standard broadcast bait application over the entire area was utilized to reduce fire ant populations. Fire ant populations were reduced by 70% after this treatment. Monitoring and targeted bait applications may be an efficient method to maintain tolerable levels of fire ants, once excessive populations have been initially reduced with a standard broadcast bait application. Analysis of different thresholds for a spatial surface generator suggested that the threshold of 1 fire ant in the MABs resulted in maps that encompassed most of the mound locations. Using thresholds of 25 and 100 ants in the MABs did not adequately indicate mound locations.

BAIT DISTRIBUTION AMONG MULTIPLE COLONIES OF PHARAOH ANTS (HYMENOPTERA: FORMICIDAE)

D. H. Oi, K. M. Vail, and D. F. Williams

Objective: The Pharaoh ant, *Monomorium pharaonis*, is a cosmopolitan, structure invading pest ant, that can mechanically spread pathogens and become an exasperating nuisance. Pest control operators have reported that rapid reinfestations of these ants can occur after treatments with ant baits that contain a metabolic inhibiting (MI) toxicant. It was speculated that colonies killed by this type of toxicant, are quickly replaced by other colonies. Another type of ant bait toxicant is the insect growth regulator (IGR), which eliminate ant colonies more slowly by sterilizing queens and killing immature ants. Because IGRs do not quickly kill existing adult ants, we hypothesized that IGR ant baits would be distributed more thoroughly among several colonies, and thus be more effective in preventing reinfestations. Our objective was to compare the extent of MI and IGR ant bait distribution and their effect on Pharaoh ant populations living in colonies located in several nest sites.

Methods: The distribution and efficacy of an MI and an IGR ant bait were compared among groups of four colonies of Pharaoh ants. The MI bait contained the active ingredient, hydramethylnon, and the IGR bait contained pyriproxyfen. Both active ingredients were dissolved in peanut oil and absorbed onto a corn grit carrier. Pharaoh ant colonies were placed in separate sections of a foraging arena. The foraging arena consisted of 8 connected trays, which resulted in a 4.7 m long arena. Colonies were placed in alternate trays starting with the trays at each end. Dyed bait (2 g) was placed in one end tray and an alternative source of food (2 g) was placed in the other end tray. As a result, colonies were located at distances of 15, 132, 310, and 427 cm from the bait, and at reciprocal distances from the alternative food source. The dye

in the bait allowed the distribution of the bait to be followed among the nest sites. The number of live, worker caste adults, live queens, and the amount of brood (immature ants) were determined for each nest site at weekly intervals for a total of 8 weeks.

Results: Within 3 weeks, the hydramethylnon bait reduced worker and brood populations by at least 80%, and queen reductions ranged between 73 and 100%, when nests were in close proximity (within 132 cm) to the bait source. However, these nest sites were re-occupied by ants from other colonies located further from the bait source. The pyriproxyfen bait was distributed more thoroughly to all nest locations with worker populations gradually declining by 73% at all nest sites after 8 weeks. Average queen reductions ranged from 31 to 49% for all nest sites throughout the study. Even though some queens survived, brood reductions were rapid in the pyriproxyfen treatment, with reductions of 95% at all locations by week 3. Unlike the metabolic inhibitor, the IGR did not kill adult worker ants quickly, thus, more surviving worker ants were available to distribute the bait to all colonies located at different nest sites. Thus, from a single bait source, the slow-acting bait toxicant provided gradual, but long-term control, while the fast-acting bait toxicant provided rapid, localized control, for a shorter duration. This study should be useful to the pest control industry because it demonstrated the importance of considering the mode and speed of action of the bait toxicant in the selection of ant baits.

BIOCHEMICAL MECHANISMS RESPONSIBLE FOR CYPERMETHRIN RESISTANCE IN THE GERMAN COCKROACH

S. M. Valles and K. Dong

Objective: Pyrethroid insecticides have been used extensively to control the German cockroach, and, as a result, resistance to this class of insecticides appears to have become prevalent among German cockroach populations. Target site insensitivity (*kdr*) has been reported to be a major mechanism of pyrethroid resistance in many German cockroach strains. However, the overwhelming majority of reports concerned with elucidating German cockroach insecticide resistance mechanisms have concluded that multiple resistance is a common motif in this insect. Knowledge of the contribution that each mechanism plays in the overall resistance level is essential to a complete understanding of the resistance phenomenon. The purpose of this investigation was to examine the mechanisms responsible for cypermethrin resistance in a recently collected population of German cockroach.

Methods: The German cockroach strain (designated the Aves strain) characterized in this study was collected by vacuum from a single family home in Gainesville, Florida, on 11 June 1998. Insecticide bioassays, detoxification enzyme assays, quantitative and qualitative cypermethrin metabolism assays, cuticular insecticide penetration rate, and evaluation of the *para* gene for the Leu993Phe mutation were conducted on the Aves strain and compared with a standard insecticide susceptible strain of German cockroach.

Results: Topical bioassay data revealed that the Aves strain was highly resistant to cypermethrin, exhibiting a resistance ratio of 93-fold which was reduced to 29-fold when cockroaches were pretreated with piperonyl butoxide and 18-fold when pretreated with S,S,S-tributyl

phosphorotrithioate. The synergist data implicated enhanced oxidative and hydrolytic metabolism as resistance mechanisms in the Aves German cockroach strain. This conclusion was further supported by significantly higher oxidative (2.4- to 4.2-fold) and hydrolytic (1.6- to 3.6-fold) detoxification enzyme activities toward surrogate substrates and significantly higher *in vitro* [¹⁴C]cypermethrin metabolism. Microsomal NADPH-dependent (1.8-fold) and NADPH-independent (2.2-fold) [¹⁴C]cypermethrin metabolism were significantly greater in the Aves strain as compared with the Orlando insecticide susceptible strain. *In vivo* penetration studies with [¹⁴C]cypermethrin indicated that decreased cypermethrin penetration may also be a contributing resistance mechanism in the Aves strain. Finally, the Leu993Phe mutation shown previously to be associated with knockdown resistance (*kdr*) was present in the Aves strain.

Figure 1. Percentage of [¹⁴C]cypermethrin Metabolized *In Vitro* by Insecticide-resistant (Aves) and -susceptible (Orlando) German Cockroach Strains

Strain	Cofactor	Synergist/ inhibitor ^a	Subcellular fraction	Tissue ^b		% Cypermethrin metabolized/30min (±SE)
				Source	Quantity (mg)	
Orlando Aves	---	---	Soluble Fraction	Whole body	0.5	35.8 ±1.4
	---	---	Soluble Fraction	Whole body	0.5	34.8 ±1.8
Orlando Aves	NADPH	DEF	Microsomes	Whole body	0.5	11.6 ±1.8
	NADPH	DEF	Microsomes	Whole body	0.5	21.8 ±1.9*
Orlando Aves	---	---	Microsomes	Whole body	0.5	13.8 ±2.0
	---	---	Microsomes	Whole body	0.5	30.4 ±4.5*
Orlando Aves	---	DEF	Microsomes	Whole body	0.5	3.8 ±1.5
	---	DEF	Microsomes	Whole body	0.5	2.1 ±1.2

^a DEF, S,S,S-tributylphosphorotrithioate (0.1 mM).

^b Whole body, entire cockroach (without the head) used as the tissue source.

^c Mean percent recovery was 92.6 ±0.8%; values followed by an asterisk are significantly different ($P < 0.05$) from the Orlando strain by Student's t-test.

COMPARATIVE INSECTICIDE SUSCEPTIBILITY AND DETOXIFICATION ENZYME ACTIVITIES AMONG PESTIFEROUS BLATTODEA

S. M. Valles, P. G. Koehler and R. J. Brenner

Objective: Although some biological control agents and related biorational methods are available for controlling peridomestic cockroach species, insecticides remain the principal control method, especially among domestic species. Despite nearly complete reliance on insecticides for cockroach control, sparse comparative insecticide susceptibility data are available for the most persistent cockroach pests. To gain a more complete understanding of the response of Blattodea to insecticides, we quantified the susceptibility to 3 insecticide classes and measured three detoxification systems, microsomal oxidase, esterase, and glutathione S-transferase, in 8 of the major cockroach pests of humans.

Methods: *Blattella asahinai* Mizukubo (Blattellidae) were collected from June to August 1986 from Tampa and Lakeland, Florida. The *Blattella vaga* Hebard (Blattellidae) colony was started from stock obtained from cultures at the University of California, Riverside, California. *Blattella germanica* (L.) (Blattellidae) was the standard insecticide-susceptible strain (Orlando strain) described by Koehler and Patterson. *Periplaneta australasiae* (F.) (Blattidae), *Periplaneta americana* (L.) (Blattidae), *Blatta orientalis* (L.) (Blattidae) and *Periplaneta brunnea* Burmeister (Blattidae) were collected from Florida and Georgia sometime before 1967. The *Periplaneta fuliginosa* (Serville) (Blattidae) colony was started in 1986 with individuals collected from Gainesville, Florida.

None of colonies had been exposed to insecticide selection pressure while in culture. Insecticide toxicity was assessed by topically applying insecticide to the cockroaches. Adult male cockroaches of unknown age were anesthetized with CO₂ (15-20 sec

exposure), placed into a Petri dish and treated topically with insecticide dissolved in 1 µl of acetone.

Results: Based on lethal dose values, the relative toxicities of the insecticide classes were generally pyrethroid > carbamate > organophosphorous. λ-Cyhalothrin and propoxur were more toxic toward the Blattidae as compared with the Blattellidae. The order of λ-cyhalothrin toxicity was *Periplaneta americana* > *Periplaneta brunnea* = *Periplaneta australasiae* = *Periplaneta fuliginosa* = *Blatta orientalis* > *Blattella asahinai* = *Blattella germanica* > *Blattella vaga*. The order of propoxur toxicity was *B. orientalis* > *P. americana* > *P. brunnea* = *P. australasiae* > *B. asahinai* > *P. fuliginosa* = *B. germanica* > *B. vaga*. The order of chlorpyrifos toxicity was *P. americana* > *B. asahinai* = *B. vaga* > *B. orientalis* = *P. australasiae* = *P. brunnea* > *B. germanica* = *P. fuliginosa*. Detoxification enzyme activities for each species also were measured and compared with insecticide toxicity. Propoxur LD₅₀ was significantly ($P = 0.01$; $r = 0.81$) correlated with glutathione S-transferase activity. λ-Cyhalothrin LD₅₀ correlated with methoxyresorufin O-demethylase activity ($P = 0.01$; $r = 0.81$), carboxylesterase activity ($P = 0.03$; $r = -0.75$), general esterase activity ($P = 0.02$; $r = -0.79$), and cockroach weight ($P = 0.01$; $r = -0.95$).

PRECISION TARGETING: REDUCED PESTICIDE USE STRATEGY FOR PHARAOH'S ANT (HYMENOPTERA: FORMICIDAE) CONTROL

D. F. Williams, R. J. Brenner, and D. Milne

Objectives: The Pharaoh's ant, *Monomorium pharaonis*, is cosmopolitan in its distribution having been carried by commerce to inhabit all regions of the earth. This ant is a major indoor pest in the United States and many parts of the world and infests almost all areas of a building principally where food is routinely available. They are one of the most difficult indoor pests to control. This study was designed to determine: 1) whether spatial statistical analysis could be used to document the impact of toxic ant baits placed on exterior window sills of a facility on the number of Pharaoh's ants foraging inside the building (routine standardized, monitoring), and 2) whether population distributions for ants foraging on the outside window sills could accurately predict interior infestations.

Methods: The study site was the Bachelor Officers Quarters (BOQ) building at the U.S. Naval Air Station, Jacksonville, Florida. The building (7,841 m²) is a two-level structure with 4 separate wings on the ground floor and 3 wings on the second floor. The study was initiated in 1996 and ended in 1997. Monitoring consisted of baiting 249 exterior ground floor window sills to determine Pharaoh's ant foraging distribution. A liquid multiple ant bait (MAB) was placed on the exterior window sills of the building for 1 hour, then retrieved and the number of foraging Pharaoh's ants on each was recorded. Monitoring and treatments were performed for 2 months on the exterior of the building and in 1997 both the exterior and interior floors were monitored. Spatial analysis was applied to the data using ArcView Geographic Information System (GIS) (ver.3.1, Environmental Systems Research Institute, Redlands, CA, 92373 USA). As a measure of risk assessment, MAB counts were reduced

to presence or absence. These data were then geostatistically gridded using the inverse distance weighted method and the resultant grid values provided contour lines ranging in values from 0 to 1 representing probabilities of detecting foci of ant foraging. Sites positive for foraging ants were then treated with Combat Superbaits®. Probability contour maps of population distribution were generated to assess success of treatments.

Results: Numbers of foraging ants were lower 1 month post initial treatment, and continued to drop showing an 88% reduction in foraging ants by the 2nd month. A survey at this time of the interior of the building showed only 3 locations positive for foraging ants. Monitoring showed that the exterior treatment of this location eliminated the interior population for the remainder of the study. Three months post initial survey there was a population increase of foraging ants on the exterior of the building with 14 positive sites. Of these 14 sites, only one spatially correlated to an interior foraging population. Exterior treatments again eliminated this population. During the final survey of the exterior of the building, only 1 Pharaoh's ant was found with no interior corresponding population. This study confirmed that the vast majority of populations present inside the structure could be eliminated with exterior bait treatments.

COCKROACH EXTERMINATION DOES NOT RAPIDLY REDUCE ALLERGEN IN SETTLED DUST

L. W. Williams, MD¹, Patrick Reinfried, BA¹, and R. J. Brenner

Objective: Asthmatic children are at high risk of cockroach-induced asthma resulting from exposure to allergenic cockroach proteins. Medical practitioners have theorized that exterminating the cockroaches will reduce exposure to allergens. However, because allergenic cockroach proteins may persist for many years in a structure, eliminating cockroaches may only prevent further accumulation of allergens, but would not address allergens that had accumulated over time. Therefore, we hypothesized that eliminating cockroaches would not reduce allergen loads significantly.

Methods: The impact of cockroach management on allergen load was studied in 8 homes near Durham, NC over a 6 month period. Sticky traps left in place overnight were used to measure populations of German cockroaches, *Blattella germanica*. Sticky traps were used and revealed substantial German cockroach populations. These traps were used to guide placement of bait stations containing hydramethylnon. Multiple dust samples from 1m² vacuumings (2 min. each), taken at 2 month intervals, were analyzed using monoclonal antibodies (units per ml of Bla g 1 and Bla g 2 were converted to units of allergen per gram of dust). Homes were assigned to active intervention (n=5) or to placebo (n=3) in a single blind fashion. We placed 2% hydramethylnon bait trays (Combat Superbait, Clorox Company, Pleasanton, CA) in actively-treated houses and identical-appearing placebo bait stations

in placebo-treated houses. Homes were visited again at 2, 4, and 6 months and were re-sampled fully each time.

Results: At 2 months, 4 of 5 treated homes showed a greater than 95% reduction in cockroaches trapped ($p < 0.05$, Wilcoxon rank-sum test), and this was sustained through the 6 month follow-up. Sufficient dust for analysis was obtained from all the rooms. At 2 and 4 months, no significant difference was seen between active and placebo homes for either Bla g 1 or Bla g 2 content in the dust. At 6 months, in the treated homes, there was a statistically significant decrease in Bla g 1 ($p = 0.048$, Wilcoxon rank-sum test) but not Bla g 2. We conclude that despite the reduction in cockroaches present, there was not a correspondingly impressive decrease in allergen in the dust. The statistical effect on Bla g 1 at 6 months is trivial, because it resulted from the presence of minimally higher Bla g 1 in the 2 remaining placebo homes than in the hydramethylnon-treated homes. The allergen content at 6 months of treatment is not a clinically significant fall in allergen levels if no special cleaning efforts are made. We concluded that elimination of existing allergens must be accomplished concurrent with cockroach control measures. Currently, the extent of cleaning required to effect an immediate drop in allergen is not known, and additional research is underway.

¹ Duke University Medical Center

MOSQUITO

AND

FLY

CRIS - 6615-32000-031-00D--Repellent Systems and Control Strategies
for Mosquito/Vectors of Medical and
Veterinary Importance

CRIS - 6615-32000-032-00D--Biological Control and Integrated
Management of Bloodsucking and
Nuisance Flies of Med/Ag/Vet
Importance

OPTIMIZATION OF RELEASE RATES FOR A TWO COMPONENT BLEND THAT ATTRACTS *Aedes aegypti*

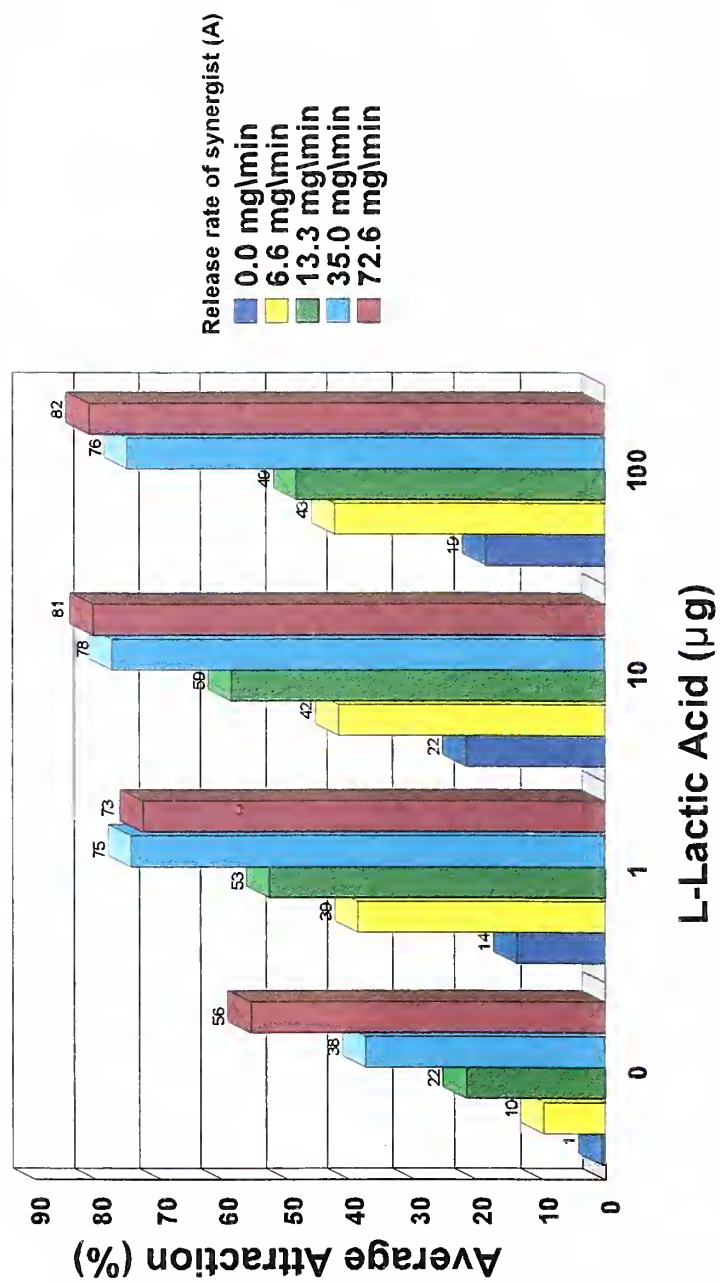
U. R. Bernier, D. L. Kline, K. H. Posey, and D. R. Barnard

Objective: The attraction of Yellow Fever mosquitoes to L-lactic acid and a synergistic compound (A) was examined. The compounds were tested alone and in combination, using various release rates, to ascertain whether an optimum ratio exists over the ranges studied. These experiments were conducted to examine if the ratio of these attractants from the skin has an optimum, if the attraction of the combination will plateau at the high release rates, and if a change in emission rate of one the compounds will have a greater impact than the other on attraction to the blend.

Methods: A triple cage, dual port olfactometer was used to assess the attraction of 6-8 day old laboratory reared nulliparous female *Aedes aegypti*. The temperature and humidity were controlled and set to 80 °F and 60% relative humidity. Bioassays were conducted as three minute tests, three times a day, at 08:30, 11:00, and 13:00 hours. Prior to tests, cages were loaded with approximately 75 hand-drawn female mosquitoes; the mosquitoes were allowed to acclimate for approximately one hour before testing. The number of mosquitoes trapped in the baited and unbaited ports, and those remaining in the cage were counted. The data were recorded as a percentage of total mosquitoes in the cage that were attracted to the baited port. L-lactic was deposited onto 14 mm i.d. vial cap at doses of 1, 10, and 100 µg (a blank vial cap was used for 0 µg) from a methanolic L-lactic acid solution. The methanol was allowed to evaporate off for 3 min prior to testing. The synergist release rates were calculated to be 6.6, 13.3, 35.0, and 72.6 mg/min (an empty glass insert was used for a 0.0 mg/min release rate). The second port

contained the same apparatus as the baited port, except that the chemical or blends were omitted.

Results: Experimental results show that various chemicals can be substituted in place of carbon dioxide, and when combined with L-lactic acid, result in synergistic attraction of *Ae. aegypti*. The two component blend described here averages 88% attraction when 200 µg L-lactic acid is combined with a high release rate of the synergist. However, many of the mosquitoes, although highly attracted, do not fly to the back of the trap in the olfactometer without first landing in the trap cone and crawling in. This behavior is dissimilar to that observed when human odors are used for attraction. The difference in behavior is believed to result from an excessively high release rate of synthetic chemicals. When odors from a live host are tested, mosquitoes tend to fly through the cone with little difficulty, collecting at the back of the trap which is nearest the hand and arm. **Figure 1** indicates that there is a plateau effect for high release rates of synergist and L-lactic acid. The base attractant, L-lactic acid, exhibits a constant attraction level for tests with 10 µg or more. This amount is thought to be below physiological levels and implies that as long as some L-lactic acid is present along with a synergist, mosquito attraction will occur. The benefit from the presence of the synergist is more pronounced and a plateau is observed for average release rates of 35.0 mg/min or more. The necessity to have an emission rate above physiological levels for efficient attraction indicates that additional important compounds are missing. Work is underway to identify other human-produced compounds that are important for mosquito attraction.



A MECHANICAL BARRIER FOR PREVENTING CLIMBING BY LESSER MEALWORM (COLEOPTERA: TENEBRIONIDAE) AND HIDE BEETLE (COLEOPTERA: DERMESTIDAE) LARVAE IN POULTRY HOUSES

C. J. Geden and D. A. Carlson

Objective: Litter beetles, including the lesser mealworm and the hide beetle, have emerged as the most important arthropod pests affecting poultry production worldwide. Larvae tunnel into building insulation materials and structural timbers, resulting in costly damage. The beetles also are reservoirs of many avian pathogens. Annual losses to litter beetles have been estimated at \$16 and \$10 million in Virginia and Georgia, respectively. There are no effective conventional control methods for these destructive insects. Our objective was to evaluate mechanical barriers to prevent larvae from climbing the walls and support posts of poultry housing.

Methods: Initial laboratory tests indicated that beetle larvae of both species were unable to cross a 6 inch strip of plastic of various types. For field evaluations the barrier tested was composed of polyethylene terephthalate plastic (PET, type "G") that was attached in "collar" form to posts or in continuous strips to walls using all-purpose caulking. Barriers were installed in a commercial high-rise pullet house in Brooker, Florida with 100,000 birds in November of 1997. Emigrating beetles were monitored by weekly placement of 76-cm lengths of 20-cm wide corrugated cardboard in a collar around the posts and by direct visual counts of beetles below *versus* above the barriers. Fly populations also were measured, and the effect of fly deposition of fecal spots on the barriers was assessed. To determine whether fly spot removal would restore the efficacy of the barriers, half of the barriers were washed with water after a fly outbreak had occurred in November of 1998.

Results: In preliminary laboratory bioassays, no lesser mealworm larvae were able to cross the plastic barriers and enter the cardboard traps. Similar results were observed with barriers held in a caged layer poultry house for three months, indicating that normal feed, manure and bird dust accumulations did not compromise the effectiveness of the plastic as a beetle barrier. The addition of a teflon dispersion to the plastic had no effect on the effectiveness of the barriers. When field-installed barriers were challenged by natural populations of litter beetles, the barriers were over 99% effective against beetle larvae and adults as determined by cardboard traps placed above or below the plastic in the weeks immediately after installation. One year after installation the barriers remained >94% effective. Barrier effectiveness decreased somewhat during a fly outbreak in November 1998, with a decrease in barrier efficiency from 97.3% to 87.9% occurring when accumulated fly spotting on the plastic exceeded 31 fly spots per cm². Removal of fly spots with water restored the efficiency of the barriers to >99% compared with 83% efficiency for unwashed posts. Barriers attached to the walls were also highly effective, and prevented an average of 2,488 litter larvae per week from crossing the barrier. A patent application for the barrier has been filed (Serial No. 09/216,513) and a manuscript on this work prepared for publication in J. Econ. Entomol.

An Olfactometer Evaluation of Several Candidate Compounds As Inhibitors of *Aedes aegypti* L. Host Seeking Behavior

D. L. Kline, U. R. Bernier and D. R. Barnard

Objective: The overall objective of this research thrust is to develop repellents for the protection of humans and animals from biting arthropods. The specific objective of this project was to evaluate the efficacy of Deet, dehydrolinalool and linalool as behavioral inhibitors of the host seeking capability of *Aedes aegypti* in a laboratory olfactometer.

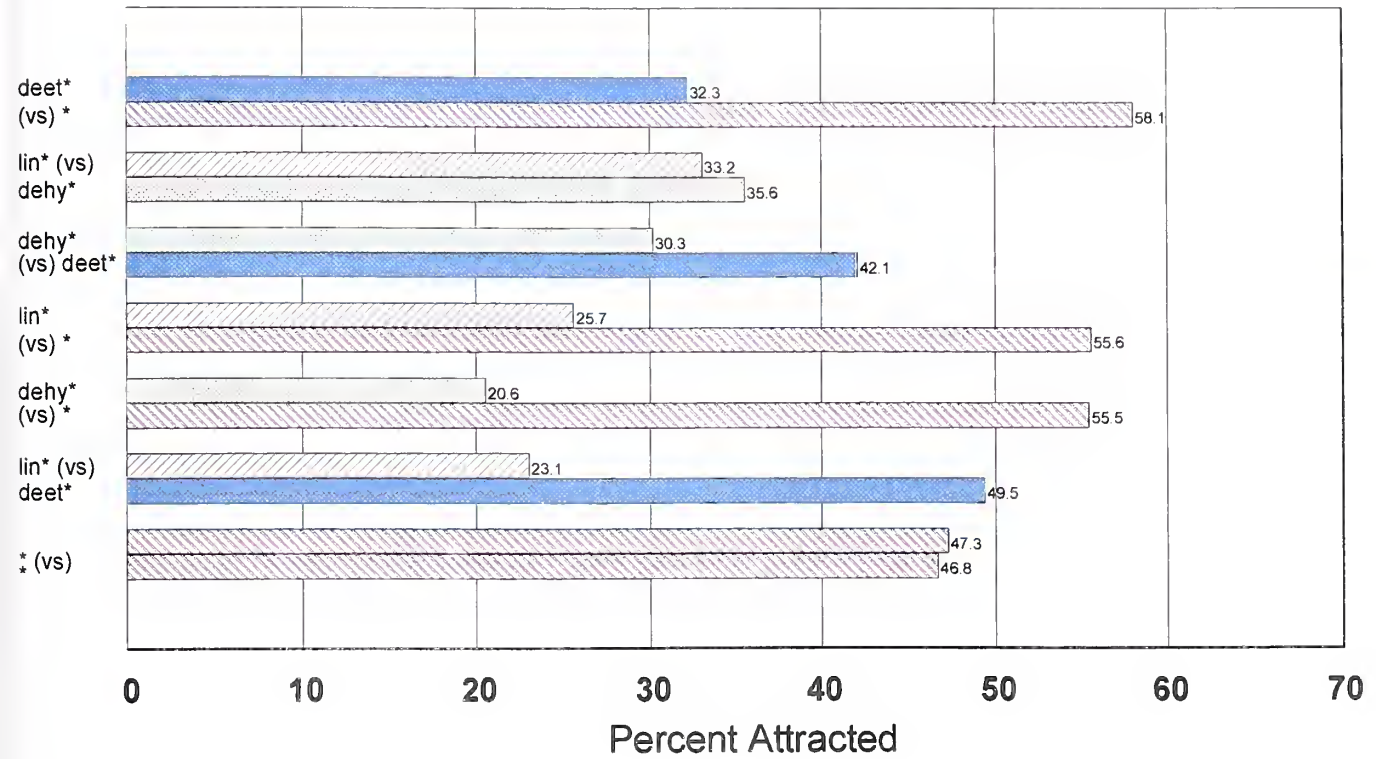
Methods: A triple cage, dual port olfactometer system was used to evaluate mosquito responses to these candidate compounds. This system allows mosquitoes to choose between two different stimuli. In this study the stimuli consisted of a choice between a port containing the candidate inhibitor plus a human odor mixture placed in separate petri dishes and another port containing only the human odor. In all cases 500µl of the human scent mixture was used. The candidate inhibitors were tested at 3 different dosages (100, 250 and 500µl). During olfactometer operation, only 1 test cage was used at a time. Each test cage contained 75, 6- to 8-day-old female mosquitoes. A completely randomized design was used to assign treatment combinations to the various cages and test periods. Each test consisted of a 3 minute exposure period, after which the number of mosquitoes caught in each port trap was recorded.

Results: Since the general behavior trend was similar for each compound for all 3 dosage levels, this summary only includes the data for the 500µl level (Figure 1). It was observed that a sequence of events occurred in the host seeking behavior of these mosquitoes: activation off the back screen of the mosquito holding chamber screen, orientation toward the ports containing the

candidate compounds, entry into the mosquito traps found at each port and attempts to probe the front screen of these traps. As a means for comparing the effect of these compounds on host seeking responses one treatment consisted of 500µl of the human scent mixture in each port. The results of this comparison showed that >90% of the mosquitoes were activated off the back chamber screen. There was a nearly 50:50 collection in each port trap. When Deet + human scent versus human scent alone was tested, ca. 90% of the mosquitoes became activated and orientated toward the traps. Approximately 64% of those responding entered the human scent only trap compared to 36% in the Deet + human scent trap. When dehydrolinalool was used, only 76% of the mosquitoes were activated off the backscreen and oriented toward the ports containing the candidate compounds. Approximately 73% of the responding mosquitoes entered the human scent mixture trap only. When linalool was used, ca. 81% of the total mosquito population in the cage was activated off the backscreen and oriented toward the ports. Of these 68% entered the human scent only port. The comparison between dehydrolinalool + human scent versus linalool + human scent resulted in only 69% activation of the mosquitoes.

500 ul dosages

Olfactometer Tests With *Aedes aegypti*



* = 500ul of Attractant in 60X15mm petri-dishes.

All other chemicals tested at full strength dosages at 500ul in 60X15mm petri-dishes.

EFFECT OF PARTIAL BLOOD ENGORGEMENT AND PRE-TEST CARBOHYDRATE AVAILABILITY ON THE REPELLENCY OF DEET TO *AEDES ALBOPICTUS*

R. Xue and D. R. Barnard

Objective: To determine if repellent protection time against *Aedes albopictus* is affected by carbohydrate availability and partial blood engorgement when deet was applied to human skin and tested against caged populations of mosquitoes. Findings will be used to standardize repellent bioassays against *Ae. albopictus* and may be useful for revision of ASTM standard E951-94, which presumes the use of nulliparous mosquitoes but does not specify carbohydrate availability during the pre-test period.

Methods: Mosquito attack rates and responses to deet were evaluated in 64,000 cm³ metal/screen cages (Experiment 1) and in 80 cm³ plastic cages (Experiment 2). The latter test arena approximated the space and mosquito density conditions defined in the ASTM standard. **Experiment 1.** A factorial design was used to make three tests of four treatment combinations comprising 10% sugar water/no sugar water with either a partial or no blood meal in the mosquito. Mosquito attack rate was determined by exposing the mosquitoes in a cage to an untreated arm for 1 min and recording the numbers that probed the skin. Repellency was determined by the same process except that a deet treated arm was observed for 3 min. Observations were repeated every 30 min. The test for a cage ended when the cumulative number of probes reached 3. Repellent protection time was that elapsed between deet application and the end of the test. **Experiment 2.** The treatment conditions cited above were used in

Experiment 2 except that mosquitoes were placed in individual plastic cages. The attack rate was determined by holding the cage against untreated forearm skin for 1 min, and observing for a probing response; repellency was determined by holding the cage against a repellent treated forearm for 3 min and observing probing responses.

Results: Experiment 1: Mosquito attack rates were higher in the water only group compared with the sugar only group. Mosquitoes with pre-test access to sugar water took the longest to bite repellent treated skin, regardless of blood engorgement state, whereas deet was repellent to partially blooded females longer than to non blood fed females, regardless of sugar availability. Experiment 2: Attack rates were highest in sugar starved females and lowest in partially blood fed females, whereas deet repellency was longest to mosquitoes that had access to sugar, regardless of blood engorgement status. From these results we concluded that (a) mosquitoes with access to sugar solution and to blood before a repellents test are repelled longer by the same dose of deet than mosquitoes receiving neither sugar nor blood, (b) that blood engorgement status affected repellent protection time in large, but not small, cages, and (c) that repellent protection time was longest overall in small cages. Our results indicate a need to control pre-test conditions for mosquitoes if laboratory bioassays for insect repellency are to be reliable and support amendment of the ASTM standard to specify pretest carbohydrate availability conditions.

FIRST REPORT OF A *HELICOSPORIDIUM* FROM BLACK FLIES

J. J. Becnel

Objective: To isolate and identify new microbial pathogens from medically important Diptera that can be developed as novel control agents.

Methods: Black fly larvae were collected from Hatchet Creek, Alachua County Florida in the Fall of 1998. Larvae from the sample were examined for signs of infection and for species identification. The total number of larvae and the percentage infection was estimated from the samples. Infected larvae were prepared for ultrastructural examination by fixing dissected abdomens in 2.5% glutaraldehyde for 2 hr, postfixing in 2% osmium tetroxide, dehydrating in ethanol series and embedding in epon-araldite. Thin sections, stained in uranyl acetate and lead citrate, were photographed at 75 kV. Spores from black flies were fed to starved first instar larvae of *Helicoverpa zea*. Spores from *H. zea* were fed to *Anopheles albimanus*, *An. quadrimaculatus*, *Culex nigripalpus* and *Cx. quinquefasciatus* larvae at a dose of 1×10^4 spores/larva.

Results: A species of *Helicosporidium* was isolated from larvae of *Simulium jonesi* Stone & Snoddy (Diptera: Simuliidae) in September 1998. Infection levels were low with only 3 of approximately 200 larvae infected. Cloudy

areas in the posterior regions of the abdomen identified infected individuals. Examination of fresh tissue with phase contrast microscopy revealed oval spores that measured $6.5 \pm 0.2 \times 6.0 \pm 0.2 \mu$. In ultra-thin sectioned material, the spores contained 4 cells enclosed within a spore wall. Three of these cells are located centrally with the forth cell forming a "ring" cell or filament encircling the contents which is characteristic of previously described species of helicosporidia. Spores from black flies fed to starved *H. zea* larvae infected 20% of the individuals. Of the mosquitoes tested, only *An. quadrimaculatus* was susceptible with 60% infection levels in the exposed larvae. Spores developed primarily in the haemocoel of *An. quadrimaculatus* without significant immature mortality. Large numbers of spores can be produced in *H. zea* larvae which maintain their viability after freezing.

A NEW CYTOPLASMIC POLYHEDROSIS VIRUS FROM CHIRONOMIDS

J. J. Becnel and T. Fukuda

Objective: To isolate and identify new microbial pathogens from medically important Diptera produced in agricultural wastewater that can be developed as novel control agents.

Methods: Chironomid larvae were collected from a man made settling pond of swine effluent located in Gainesville Florida. Samples were collected once or twice a week during the peak breeding periods and at least once a month during the off season. Larvae from the sample were examined for signs of infection and for species identification. The total number of larvae, the proportion of each species and the percentage infection was estimated from the samples. Infected larvae were prepared for ultrastructural examination by fixing dissected guts in 2.5% glutaraldehyde for 2 hr, postfixing in 2% osmium tetroxide, dehydrating in ethanol series and embedding in epon-araldite. Thin sections, stained in uranyl acetate and lead citrate, were photographed at 75 kV.

Results: A new cytoplasmic polyhedrosis virus (CPV) was isolated from the larval stages of *Ablabesmyia* sp. Johansson (Diptera; Chironomidae) collected in July 1998. Infected individuals were recognized by a chalky white color in the anterior regions of the larval midgut. This species was relatively rare and of 20 specimens examined, 2 exhibited signs of infection.

Examination of fresh tissue with phase contrast microscopy revealed small granular particles restricted to the cytoplasm of the midgut epithelial cells. In ultra-thin sectioned material, free virions and virions within spherical and cuboidal occlusion bodies were loosely distributed within the cytoplasmic matrix of midgut epithelial cells. Free virions were formed within a granular, amorphous material believed to be virogenic stroma. Occluded virions (approximately 50nm) were more compact and dense and were usually surrounded by a distinct electron lucent halo. With few exceptions, occlusion bodies contained a single, centrally located virion. It appeared that the occlusion bodies were first spherical in shape and as they matured they grew in size becoming more angular and finally cuboidal. The typical mature occlusion bodies were approximately 200nm in diameter but rarely, larger occlusion bodies with 2-3 virions were formed. The occlusion bodies exhibited a macromolecular paracrystalline lattice arrangement characteristic of polyhedrin protein. There was no evidence of an envelope surrounding the occlusion bodies.

CHARACTERISTICS OF A NEW BACULOVIRUS FROM THE MOSQUITO *CULEX NIGRIPALPUS*

J. J. Becnel, S. White, B. Moser, T. Fukuda and M. A. Johnson

Objective: To isolate and characterize new microbial pathogens from medically important Diptera produced in agricultural wastewater that can be developed as novel control agents.

Methods: Mosquito larvae were collected from a man made settling pond of swine effluent located in Gainesville Florida. Samples were collected from September 1996 through January 1998 once or twice a week during the peak mosquito breeding periods and at least once a month during the off season. Larvae from the sample were examined for signs of infection and for species identification. Infected larvae were prepared for ultrastructural examination by fixing dissected guts in 2.5% glutaraldehyde for 2 hr, postfixing in 2% osmium tetroxide, dehydrating in ethanol series and embedding in epon-araldite. Thin sections, stained in uranyl acetate and lead citrate, were photographed at 75 kV.

Results: A new baculovirus was found in the mosquito *Culex nigripalpus*. The baculovirus from *C. nigripalpus* is restricted to the nuclei of epithelial cells in the gastric caeca and midgut of larval mosquitoes. Infected cells are found in the proximal region of each gastric caecum and

the posterior midgut. Nuclei of cells in the anterior midgut and distal cells of the gastric caeca are rarely infected. Infected nuclei are hypertrophied and appear opaque to white in color due to the proliferation of occlusion bodies (OB) within the nuclei. Death of the larvae is rapid, usually within 3-4 days after exposure. Occlusion bodies, globular in shape with a diameter of approximately 400 nm, lack an envelope, which is characteristic of most other baculoviruses. Each OB contains up to 8 clearly visible rod-shaped virions with each nucleocapsid enveloped singly. The virions are approximately 200 X 40 nm and consist of a single nucleocapsid, intermediate layer and an outer envelope. The occlusion bodies have a density of 1.18g/ml as determined on a Ludox[®] gradient. SDS-PAGE analysis of the occlusion body revealed numerous proteins among which were a 29 kDa protein (the presumptive polyhedrin or equivalent) and a 97 kDa protein. Based on pulsed field gel electrophoresis and restriction digests with Eco RI, BamHI, and PstI, the genome size of CuniNPV is estimated to be 73 kb. This is the smallest baculoviral genome reported to date.

Attraction Responses of Stable Flies in a Triple Cage Olfactometer

D. A. Carlson

Objective: The stable fly *Stomoxys calcitrans* (L.) is a haematophagus insect whose economic impact as a pest of livestock is well established. Stable flies transmit a number of disease organisms, and are a pest that feeds on human beings (Newson, 1977), particularly the Florida Gulf Coast, Atlantic beaches and many inland locations. We wish to find attractants for these flies that could be used in traps to effectively reduce the numbers of flies on cattle, and would be useful where people are exposed to these biting pests. This effort is meant to be contiguous with field trial evaluations of novel traps for these insects.

We evaluated attraction responses to olfactory stimuli using a triple cage olfactometer. Host odors are an important cue in host location by blood-sucking flies, but stable fly attraction responses to human odors were previously evaluated using only wind tunnels, and then not recently. Because human skin is known to be a good attractant of stable flies, a human hand was used as a standard attractant in all experiments. Other synthetic attractants derived from the human attractant studies of CMAVE promise to provide ample candidate compounds for bioassays. Also, field observations suggested that attractive materials are produced by the flies themselves in the form of feces, and this hypothesis had never been tested in an olfactometer system.

Methods: Stable flies used in this work were from a colony cultured at CMAVE. A triple cage olfactometer system uses a conditioned, humidified air flow from a remote location that passes through dual ports into a test cage, controlled by a sliding door. Fly traps capture and retain flies that pass upstream through either port during each 6 minute test.

Results: No significant differences biases were found due to cage position or time of day, so 3 sets of 3 olfactometer tests were performed each day at 0900, 1100 and 1300. Attraction to a human hand was highest at 55 feet/minute, but somewhat lower at 25 and 95 feet/minute, and increased linearly as a function of time. Variation in fly density did not affect response. Females responded more (25%) than males (15%) at 55 feet/minute. A spike in flight activity was observed at about 3 min, but then declined for the rest of the test. Candidate synthetic attractants that gave good responses with mosquitoes were found to be totally ineffective against stable flies.

The attraction response of stable flies to conspecific feces was evaluated. Both time-response and concentration-response relationships were obtained for females exposed to cellulose sponges impregnated with fresh fly feces or filter papers treated with a chloroform:methanol (2:1) extract of fresh fly feces in 6 min tests. Feces collected on cellulose sponges and held for 28-31 days retained activity, and females were more attracted than males. However, the activity of feces extract on filter paper decreased rapidly. Polar solvent extracts of feces-contaminated cages were more attractive to females (26%) than to males (14%). This remains a challenging chemistry problem, because it is hard to imagine that human skin and dirty cage residues produce the same volatile attractive chemicals.

EFFECT OF LIGHTING INTERVALS ON EFFICACY OF ULTRAVIOLET LIGHT TRAPS FOR HOUSE FLY CONTROL IN CLOSED POULTRY HOUSES

P. L. Seaman, J. A. Hogsette and C. J. Geden

Objective: Ultraviolet light traps used specifically for house fly management in poultry houses were always of questionable value until the poultry industry changed to closed housing designs that allow light traps to function without diurnal competition from the sun and without becoming clogged with moths and other non-target insects at night. Although ultraviolet light traps have been shown to be efficacious in closed poultry houses, there is a strong desire by trap manufacturers to further increase the attraction of these traps to house flies. Because previous studies have shown house flies to be attracted to light traps that are suddenly illuminated after a period of inactivity, and because house flies are stimulated to fly by sudden changes in light intensity, we decided to compare the effects of selected periods of illumination on the efficacy of ultraviolet light traps to determine whether trap efficacy might be increased.

Methods: Three pairs of commercial ultraviolet light traps (Gardner Manufacturing, Horicon, WI) were hung 1.5 m above the floor and spaced at equal distances apart on one wall of a 90-m long closed poultry house near Brooker, Florida. Traps in each pair were spaced approximately 30 cm apart. Flies were killed by contact with electrocutor grids on traps and dead flies were collected in trays beneath the grids. One pair of traps was illuminated at all times during the test and was considered to be the standard. Each of the other traps was attached to a timer. These were set to allow the lights in each remaining pair of traps to be either on or

off, simultaneously or alternatively, for 1-hour intervals during 24-hour periods. Treatments were designed not only to create changes in light intensity but also to produce sudden illumination of traps over time. Treatments were rotated between the three locations along the wall and flies were captured during each rotation for three 24-hour periods. Numbers of captured flies were estimated by volumetric measurement (20 flies in 1 ml).

Results: The light traps under constant illumination captured the most flies during the test (mean = 674.5 flies/trap/day). This was followed by the treatment having one trap on or off at all times (mean = 461.5 flies/trap/day). The lowest number captured was in the treatment having both traps either on or off at 1-hour intervals (mean = 384.0 flies/trap/day). Thus, none of the treatments improved upon the standard treatment of constant illumination. Fly capture is known to be positively correlated with light intensity in traps of this type. Therefore, it was interesting to note that the pair of traps with one trap illuminated at all time captured more flies numerically than the pair of traps which were either on or off simultaneously. The former pair produced half of the light intensity of the latter when in operation, indicating that the frequency of illumination had some effect on attraction over and above light intensity. Testing will continue to determine whether this effect can be better defined and employed in the field.

POSTHARVEST
AND
BIOREGULATION

CRIS - 6615-43000-007-00D--Population Management of Insects to
Protect Stored Products

CRIS - 6615-43000-008-00D--Detection and Population Estimation of
Stored Product Insects

JUVENILE HORMONE AND JUVENILE HORMONE MIMICS INHIBIT AMINO-SUGAR UPTAKE AND PROLIFERATION IN AN INDIAN MEAL MOTH CELL LINE

H. Oberlander, C. E. Leach, and E. Shaaya

Objective: To evaluate an imaginal disc derived cell line as a bioassay tool for investigating juvenile hormone(JH) and JH mimics at the cellular level.

Methods: Although mimics of JH were the first Insect Growth Regulators evaluated for practical control of pest insects, in vitro studies with these agents have been very limited. Initial problems with stability and solubility were dealt with by combining juvenile hormone with a carrier protein for organ culture experiments. More recently, there has been some success in demonstrating direct effects of JH and JH mimics on established cell lines(Willis, *In Vitro: Cell. Dev. Biol.* 33, 86A, 1997; Iwabuchi, *In Vitro: Cell. Dev. Biol.* 35: 612, 1999). We utilized the PID2 cell line established in our laboratory from wing imaginal discs of the Indian meal moth. The test compounds were tested in complete Grace's medium over a 3-day culture period and cell numbers were determined by direct counts of the monolayer cultures with the aid of an inverted phase microscope. Uptake of radio-labeled GlcNAc by the cells was measured by standard scintillation counter procedures.

Results: We found that JH and JH mimics inhibited ^{14}C -GlcNAc uptake and proliferation in the wing imaginal disc-derived cell line, IAL-PID2 (see also Oberlander et al., *J. Insect Physiol.* In Press). The most consistent response obtained in our studies was inhibition of cell proliferation, in the absence of 20-hydroxyecdysone. JH-I, JH-III, methoprene, fenoxycarb, and farnesol significantly inhibited cell proliferation after 3 days of exposure of the cell line to these agents. Linoleic acid had no effect on proliferation in these cultures. In addition, fenoxycarb inhibited ^{14}C -GlcNAc uptake by the cells even when cultured in the presence of 20-hydroxyecdysone or RH-5992(tebufenozide). Thus, the PID2 cell line demonstrated responsiveness to JH and its mimics through inhibition of various processes in the presence or absence of ecdysteroids.

JUVENILE HORMONE PROMOTES THE MAINTENANCE OF LAMELLIPODIA IN A LEPIDOPTERAN CELL LINE, AND MIMICS THE EFFECTS OF SIGNALING BY LYSOPHOSPHATIDIC ACID AND EXOGENOUS PHOPHOLIPASE

D. S. Dyby, C. E. Leach, and H. Oberlander

Objective: To determine the cellular effects of juvenile hormone (JH) and cellular regulators in an Indian meal moth cell line.

Methods: Research was conducted on an Indian meal moth cell line which was established in this laboratory from dissected wing imaginal discs. This cell line has been utilized for numerous studies on ecdysteroids and ecdysteroid mimics, but as with other tissue culture systems has received little attention in terms of JH studies. The cells were maintained in an antibiotic-free Grace's medium supplemented with 10% fetal bovine serum. Experimental subcultures were maintained for 20 hours in serum free medium before exposure to JH or any of the cellular regulators were added. After incubation with the test compound the cells were returned to complete Grace's medium for observation.

Results: JH, fenoxycarb, lysophosphatidic acid (LPA), exogenous phospholipase D, bombesin (a neuropeptide), and linoleic acid, but not oleic acid, induce specific cell shape changes in the PID2 and the PID2A lepidopteran cell line. The percentage of cells that maintain broad lamellae (lamellipodia),

i.e. flattened extensions, doubles with the application of JH I, III, LPA, or PLD, from one to several days after test compounds are removed. The cells are sensitive to nanomolar concentrations of JHI. Complete Grace's medium is necessary to enhance the response to JH. This complete medium contains various growth factors, including transforming growth factor (TGF- β) and LPA. Gundersen et al. (J. Cell Science 107: 645-659, 1994) and Cook et al. (J. Cell Biol. 141(10): 175-185, 1998) found that TGF- β , and LPA, by activating the GTPase, Rho, stabilize microtubules in NIH-3T3 mammalian cells. We suggest a model whereby JH activates the signaling pathway controlled by extracellular LPA and preferentially promotes the uptake of the TGF- β superfamily, such as *dpp*, into the cells.

piggyBac TRANSPOSASE INDUCED MUTATIONS IN LEPIDOPTERA

P. D. Shirk, O. P. Perera and H. Bossin

Objective: To determine the utility of the *piggyBac* transposon as a gene vector in Lepidoptera. The yellow eye color phenotype of the *Anagasta kühniella* a strain is the result of a mutation in the locus that produces the tryptophan oxygenase enzyme. The availability of cDNA clones for the wild type tryptophan oxygenase gene from other insects provides a clear potential for utilizing this mutation as a marker for movement of a transposon vector during germ line transformation. These experiments were to test the suitability of the tryptophan oxygenase mutation of *A. kühniella* a strain as a means of detecting the movement of the *piggyBac* transposon in this moth. The *piggyBac* transposon was also tested in the Indianmeal moth, *Plodia interpunctella* as a gene vector system.

Methods: The tryptophan oxygenase structural gene (TO) from *Anopheles gambiae* under the control of the IE1 promoter (provided courtesy of N. J. Besansky, U. Notre Dame) was inserted into the *piggyBac* transposon. The *piggyBac*/IE1-TO construct was co-microinjected with a *piggyBac*/wc-hsp70-transposase helper into preblastoderm embryos of the *A. kühniella* a strain. The *piggyBac*/wc-hsp70-transposase helper was also microinjected into preblastoderm embryos of the *Plodia interpunctella* W+ strain (confirmed homozygous for the w white-eye locus).

Results: From 1504 microinjected eggs, 241 adults emerged and were mated with *A. kühniella* a strain adults. From those matings, 111 fertile matings occurred and 5 of those matings resulted in progeny with a brown eye

phenotype suggesting the presence of an active tryptophan oxygenase. Three of these brown eyed families produced progeny and were selected for homozygosity of the phenotype. Analysis of the genomic DNA from each of these families has shown that the *piggyBac*/IE1-TO is not present in any of these families. In the control experiment where the *piggyBac*/wc-hsp70-transposase helper alone was microinjected into 1200 eggs, 2 matings occurred where the brown eye phenotype was observed in the progeny. These findings suggest that the induction of the brown eye phenotype occurs because of the presence of the *piggyBac* transposase. This would also suggest that the mutation of the tryptophan oxygenase locus in the *A. kühniella* a strain is the result of the insertion of a TTAA type transposon that can be cross-mobilized by the *piggyBac* transposase.

G0 adults from preblastoderm embryos of *P. interpunctella* W+ strain that were microinjected with the *piggyBac*/wc-hsp70-transposase helper were mated with a *P. interpunctella* w- strain (white-eye strain). In 5% of the reproductive families, white-eye G1 progeny were recovered. The presence of the white-eye genotype in progeny from the *P. interpunctella* W+ strain most likely was induced by the presence of the *piggyBac* transposase.

PREVENTING FLOUR MOTH INFESTATIONS OF STORED COMMODITIES: ALTERNATIVES TO HARD PESTICIDES

D. Silhacek and C. Murphy

Objective: The increasing requirement for safer methods to control insect pests that infest stored commodities has prompted this laboratory to investigate alternative procedures that would eliminate or minimize the hazards associated with pesticide use. Our approach has been to step back and take a look at the various parameters that constitute a successful infestation of a commodity. We have broadly defined the fundamental requirements for an infestation of a food product stored in a clean warehouse as: 1) Attraction of the female insect to the commodity, 2) Oviposition of viable eggs on the commodity, 3) Successful growth and development of the insect on the commodity. Interfering with one or more of these parameters should effectively prevent the infestation of the commodity.

Approach: Our work is examining these requirements for infestation using one or more species of flour moths that infest high value, processed commodities packaged for warehouse storage. In one study, we are identifying the components required to attract female moths to a packaged commodity for oviposition. In a second study, we are designing new protocols that effectively use Insect Growth Regulators (IGRs) to interrupt insect development in packaged commodities. A third study is focusing on the nutritional adequacy of commodities for insect pests; our intent is to tailor processed commodities so that they are nutritionally inadequate for sustaining a population of infesting insects.

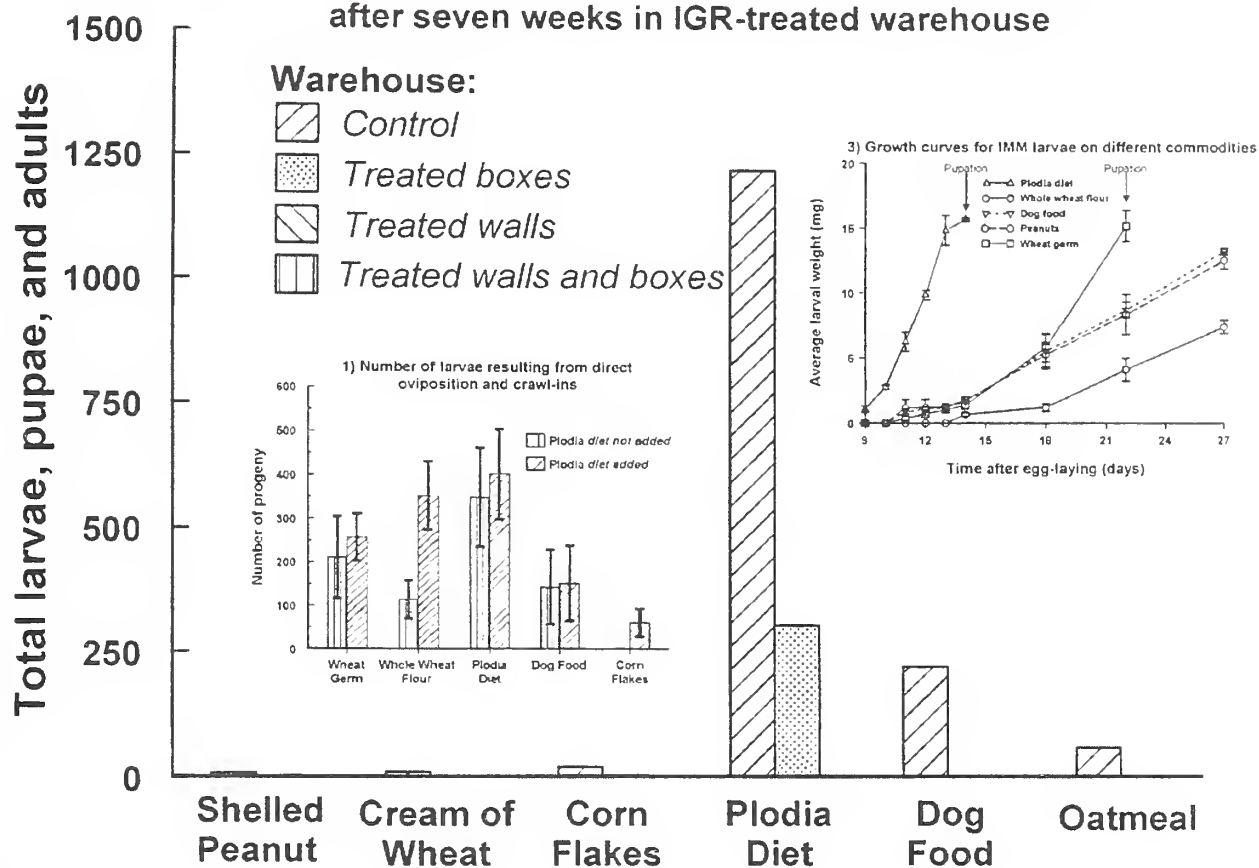
Progress: We have developed methodology that quantifies the attraction of moths to specific commodities for oviposition (Fig 1) and have readily demonstrated species differences in the attractiveness within a short

list of test commodities. We plan to proceed with the isolation of the attractive components from the most attractive commodity so that they can be identified and used in developing methodology designed for monitoring and controlling grain moth infestations of commodities during storage.

We have developed methodology for delivering IGRs to eggs that interrupts normal embryogenesis and prevents egg hatch without treating the commodity. The two most effective compounds, are the juvenile hormone mimics, fenoxycarb and pyriproxyfen. Warehouse tests have demonstrated (Fig 2) that a single application of these compounds can protect packaged commodities from cereal moth infestation for periods as long as 6 months. Development of this technology provides a safe, biorational alternative to hard pesticides.

We have shown that some cereal products are inadequate for optimum insect growth (Fig 3) and have made progress towards identifying the components in cereals that are essential for maintaining an insect infestation. As a first step, we have developed a simplified diet for rearing grain moths, which makes it simpler to isolate and identify these essential cereal components. The next step will be to determine if an essential component for insects can be removed during processing or by genetic engineering without compromising the commodity's mammalian food quality. Precedents for food products being nutritionally inadequate for insect growth are already in the marketplace, but are largely unrecognized.

2) Number of Indian meal moths (IMM) found in commodities after seven weeks in IGR-treated warehouse



MONITORING STORED-PRODUCT INSECTS IN PET STORES

R. T. Arbogast, P. E. Kendra and R. W. Mankin

Objective: Stored-product insects are a perennial problem in retail stores, where they damage and contaminate susceptible merchandise such as food products and animal feed. Effective management of the problem requires good sanitation, frequent rotation of stock, monitoring for pests, removal of infested stock, and judicious application of biorational or conventional chemical pesticides. Our objective was to test the effectiveness of trapping and contour analysis of trap counts as a monitoring method to detect and locate foci of infestation in pet stores, where stored-product insects are especially troublesome, and to evaluate the effectiveness of control intervention.

Methods: The study was conducted in two pet stores, one in Gainesville and one in Ocala, FL. Moths were monitored with pheromone-baited sticky traps (SP-Locator traps with Minimoth lures, AgriSense, Mid Glamorgan, UK), and beetles with pitfall traps (FLIT-TRAK M², TRÉCÉ, Salinas, California) baited with cigarette beetle and flour beetle pheromones and oat oil. A moth trap and beetle trap were placed at each of forty locations. Trap location was specified in rectangular coordinates with the origin at one corner of the store. Moth traps were placed 1.0 to 2.3 m above the floor and were concealed under shelves, where they were attached by means of Velcro for easy removal and replacement when making counts. Beetle traps were placed on the floor, either under a shelf or against a wall. Two trapping campaigns were conducted in the Gainesville store, one before and one after initiation of a program of sanitation and hydroprene (Gentrol®) applications. Trapping in the Ocala store began after the program was initiated. Contour analysis was done with Surfer Version 6.02 (Golden Software, Golden, Colorado).

Results: Infestations involved mainly three species of stored-product insects: the Indian meal moth, *Plodia interpunctella* (Hübner), the cigarette beetle, *Lasioderma serricorne* (Fabricius), and the merchant grain beetle, *Oryzaephilus mercator* (Fauvel). Trapping and contour analysis effectively mapped the distribution of each pest species in the stores and located foci of infestation (Fig. 1), which facilitated mitigation by cleanup, removal of infested products and application of hydroprene. The numbers of beetles and moths captured in the Gainesville store were much lower after initiation of improved pest management (cf. Figs 1A and 1B). Also, fewer beetles, but not moths, were captured in the Ocala store than were captured initially in Gainesville. At the time of trapping in Ocala, there was a serious moth infestation that had just recently become established and was traced to dog food that arrived infested from the warehouse. The beetle example shown in Fig. 1, as well as moth data for the Gainesville store, suggest that the new pest management program was effectively mitigating the pest problem. This is also suggested by the difference between the Gainesville and Ocala stores. The results show that contour analysis of trap counts provides a useful tool for management of insect pests in retail stores, and this method has recently been put into practice by at least one pest control company. The tool identifies trouble spots and permits timing and precision targeting of control measures to achieve pest suppression with minimum pesticide risk.

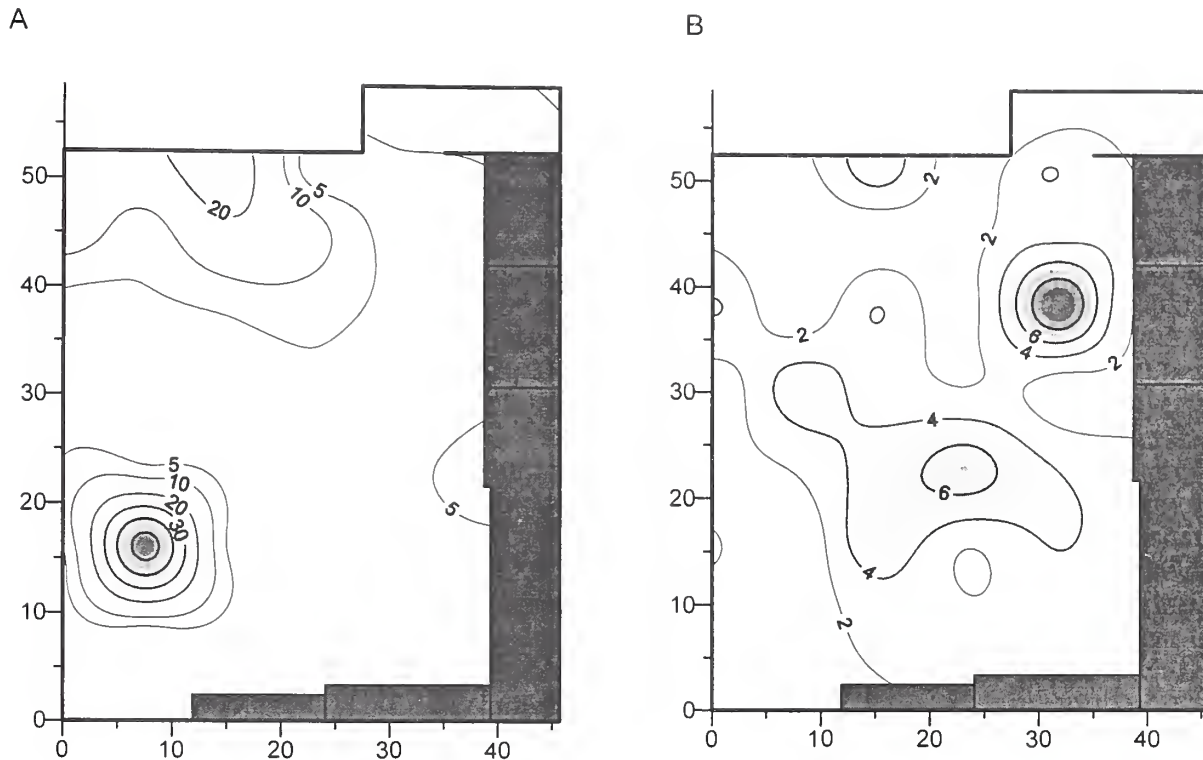


Figure 1. Spatial distribution of beetles in a pet store at Gainesville, FL as indicated by contours of numbers captured in pitfall traps during a period of 5 days. Axes indicate distance in meters from the left front corner of the store. A. Before initiation of improved pest management, including hydroprene applications. Contour interval is 10 for numbers > 10. The infestation along the back wall consisted mostly of merchant grain beetles. The isolated infestation toward the front of the store consisted mostly of cigarette beetles. B. Eight months later, and 6 months after initiation of improved pest management practices. Contour interval is 2. Note lower levels of infestation indicated by lower contour values. The low level infestation on the back wall was a remnant of the merchant grain beetle infestation. The remaining infestation consisted mostly of cigarette beetles.

ACCURACY OF A ELECTRONIC GRAIN PROBE INSECT COUNTER (EGPIC)

N. D. Epsky and D. Shuman

Objective: The Electronic Grain Probe Insect Counter (EGPIC) is a commercial grain probe trap that has been modified by adding an infrared sensor head that electronically counts insects as they fall through. Accuracy of the electronic probes is the critical component in determining the system's performance. Availability of a number of manufactured EGPIC system components allowed assessment of the factors that affect the accuracy of the electronic probes. This information can be used to further improve system accuracy.

Methods: Baseline accuracy of 54 probes was determined with drop tests using dead insects. Rusty grain beetles, sawtoothed grain beetles and red flour beetles were used for these tests, and accuracy was based on the electronic count per 100 insects. Mustard seeds (*Brassica juncea* [L.] var Florida broadleaf) were also used in some tests. Seeds were measured using an ocular micrometer with a stereoscope at 10X, and a set of 1.5-mm diam seeds was obtained. These seeds are fairly symmetrical spheres and the 1.5-mm diam was slightly less than the length of a rusty grain beetle. The output signal pulse from the phototransistor generated by a falling seed was captured with an oscilloscope (Fluke 97 Scope meter, Fluke Corp., Everett, WA) configured to give its peak value as a digital readout. The oscilloscope was used to differentiate between dropped objects that produced no output signals from those in which produced signals that were too small to trigger an electronic count, as well as to quantify the output signals. Tests of output signals were conducted with 30 mustard seeds per probe, and mV output per mustard seed drop was recorded.

Results: The accuracy of the probes increased as the size of the insect increased. The larger insects consistently pass through a large enough area of the beam to produce an output signal greater than the threshold level. When problems were encountered with accuracy of a probe, it was remedied by replacing the diode and phototransistor. Initially, it was thought that inadequate diode irradiance output and/or phototransistor sensitivity (as indicated by the size of the output signal) was the primary factor responsible for inaccurate insect counts. However, we found that probe accuracy was correlated with variation in output signal. The infrared beam intensity is not uniform throughout its cross-section, possibly due to imperfections or variations in the lens of the diode and/or phototransistor. The insect outline is irregular and orientation of the insect as it passes through the infrared beam will affect the magnitude of the output signal. An insect that presents a small profile as it falls through the beam and that passes through a weak section of the beam will produce a much smaller signal than the same insect that presents a large profile (Fig. 1). Some of this variation was removed by using the spherical mustard seeds. Even though the seeds were smaller than rusty grain beetles, accuracy in counting the seeds was higher. Variation due to irregularity in insect profile can not be reduced, however, use of diodes with a more consistent beam or modifications to improve the focus of the beam may further improve accuracy.



Fig. 1. Stop-action photographs of red flour beetles falling through the (invisible) infrared beam between two funnels in the sensor head of an electronic probe. Depending on the position when the insect crosses the beam, a small profile (left) or large profile (right) may be presented. The original photographs (USDA/ARS, Information Staff) were taken in a dark room, and the passage of the insect through the infrared beam, which triggers an electronic count of the captured insect, was used to trigger the camera.

DETECTION OF INSECTS IN PACKAGED PRODUCTS

R. W. Mankin

Objective: Food processors take considerable precautions to ensure that finished products are insect-free at the time of packaging, but there are many opportunities for insects to infest food during subsequent shipment and storage. Present methods of screening for damage between packaging and the time of receipt by a customer usually involve expensive, destructive sampling of high value-added products. However, any undetected insect contamination or damage during this period causes considerable economic loss and customer dissatisfaction. The Food Protection Committee of the Association of Operative Millers has ranked the improved detection and control of insects in packaged goods as one of the major needs of the industry. They have developed an Ad Hoc Distribution Chain Infestation Working Group to work on the problem. A major priority of the committee is a noninvasive, field-use device for pallets. To determine the feasibility of developing an acoustic device for these purposes, we conducted an initial series of laboratory experiments with bags of dog food that had been artificially infested with *Plodia interpunctella* larvae. The goal was to determine the sound levels typically present during low-level infestation and optimize the sensing system used to detect the insects.

Methods: Small bags of dog chow were infested with 20-50 *P. interpunctella* larvae and tested for detectable acoustic activity after several days of feeding. Two acoustic sensors were used to sense the infestations. One was a piezoelectric device used previously in measurements of insect activity in stored grain. The second was an accelerometer used in measurements of soil

insect activity. Only minor precautions were taken to shield the system from external noise because the system was expected to be used primarily in a noisy warehouse environment.

Results: We were able to detect and record *P. interpunctella* larval feeding activity with both types of sensor. With the accelerometer, our best results were obtained by filtering out signals above 1000 Hz and below 100 Hz. The peak signal energy occurred near 450 Hz, well within the accelerometer's frequency range of sensitivity. The piezoelectric sensor is insensitive at these frequencies, but has strong sensitivity near 2.2 kHz. We were able to amplify the signal sufficiently to pick up components between 2-4 kHz. In both cases the signals could be detected above background in an unshielded room using headphones.

Although the insects were detectable, the capability of the acoustic system would be considerably improved by better coupling between the external sensor and the food inside. The method we found to work best involved clamping or pressing the sensor as tightly as possible to the surface of the bag. This increased the effective surface area of the sensor and also improved the mechanical coupling between the insect vibrations and the sensor. Such a method might also be useful for expanding from a single bag to a whole pallet. Another possible improvement would be to establish a dual-sensor system to perform common-mode rejection analyses. These experiments are continuing, and we anticipate further improvements in the detection system.

EVALUATION OF THE ELECTRONIC GRAIN PROBE INSECT COUNTER (EGPIC) IN GRAIN STORAGE

D. Shuman and N. Epsky

Objective: A Cooperative Research and Development Agreement has been entered into with OPI Systems, Inc. of Calgary, Canada, a company that produces stored-product management systems, for development of a commercially practical system that automatically provide information about the presence and extent of insect infestations in bulk-stored agricultural products. Up to now, this information could only be obtained by visual inspections of product samples or manually deployed traps, but these methods are labor intensive and not often performed because of their difficulty and concerns for worker safety upon entering large storage bins. Automated systems will use numbers of perforated tubes distributed throughout stored-products. Insects wandering in the stored-product crawl into and then drop down past electronic sensors that send counts back to a central computer. Insect counts are automatically analyzed, resulting in displays of population distribution estimates. These displays can provide an early warning, allowing a manager to nip a local insect problem in the bud with the use of

a minimal amount of pesticide or a non-toxic alternative control measure. The real-time system data will then provide immediate feedback on the effectiveness of the applied control measure. Commercialization of this system will provide the agricultural industry with a safe, effective tool for monitoring insect populations which is an essential component of any Integrated Pest Management program.

Methods and Results: This is a new CRADA.

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